UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: **June 11, 2008**

SUBJECT: Science Review in Support of a Tolerance Exemption Petition, registration of an End-Use Product and Request for Waivers on Certain Data Requirements for Homobrassinolid and the Registration of **Homobrassinolide Technical**

Decision Number:	381556
DP Number:	347313
EPA File Symbol Number:	69361-RT
Chemical Class:	Biochemical
PC Code:	067700
CAS Number:	80483-89-2
Tolerance Exemptions:	Pending
MRID Numbers:	

FROM: Miachel Rexrode Ph.D., Senior Biologist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

THROUGH: Russell S. Jones, Ph.D., Senior Biologist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

TO: John Fournier, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

THE FOLLOWING CONTAINS CONFIDENTIAL BUSINESS INFORMATION

ACTION REQUESTED

1) Repar Corporation (Repar) has submitted a tolerance exemption petition for the active ingredient, homobrassinolide(2α,3α,22S,23S,24S)-2,3,22,23-tetrahydroxy-24-ethyl-β-homo-7-oxa-5α-cholestan-6-one), on all raw agricultural commodities (food and non-food crops including forage crops and animal feed as well as residues of homobrassinolide in meat, milk, and eggs). In addition to tolerance exemptions, the registrant is also requesting waivers for all residue chemistry, and all applicable data requirements for pre- and post- harvest uses on all crops (waiver requests for all applicable guidelines Series 171).

The registrant has also submitted data in support of the registration of the product Homobrassinolide Technical. In support of the Tolerance Exemption petition and registration, the registrant has submitted product chemistry studies, Tier I toxicity studies and waiver requests, and non-target organism studies and waiver requests.

RECOMMENDATIONS AND CONCLUSIONS

1a. Tier I Product Chemistry studies are **Unacceptable, but Ugradeable.** To update the product chemistry submission to Acceptable, the registrant must submit Storage Stability (OPPTS 830.6317) and Corrosion Characteristics studies (OPPTS 830.6320).

1b. The waiver requests for Storage Stability and Corrosion Characteristics studies were deemed unacceptable. The accelerated storage stability study submitted in fulfillment of these data requirements cannot be used in place of a one year study under normal conditions of storage and temperature (see OPPTS 830.6317 for details).

2. The following Tier I Toxicity studies and waiver requests were found to be **Unacceptable**:

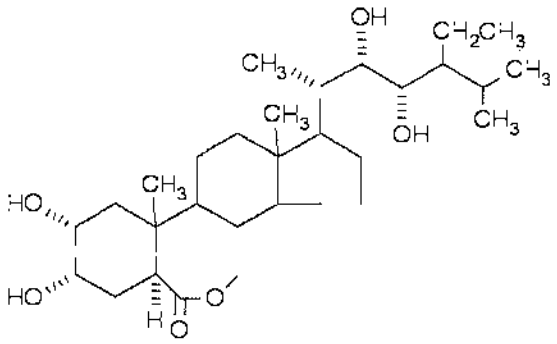
   a. Dermal Sensitization (OPPTS 870.2600); Pre-Natal Developmental (OPPTS 870.3700); and Bacterial Reverse Mutation Assay (OPPTS 870.5100). See Tier I Toxicity summaries below for details and means to upgrade studies and waiver requests to Acceptable.

STUDY SUMMARIES

Product Chemistry (MRID 47185101 – 47185117)

Brassinosteroids are a class of plant polyhydroxysteroids that are ubiquitously distributed in the plant kingdom. These compounds, when applied to plants, improve their quality and yield and have been further researched for stress-protective properties (i.e. cold, heat, salt, and heavy metal exposure). Homobrassinolide is a plant growth regulator that is proposed as controlling or

regulating the growth and development of all higher and lower plants. The proposed uses include all agronomic and horticultural crops such as agricultural crops, greenhouse food uses and non-food crops, aquatic food and non-food crops (all crops, grasses, vines, and trees listed under 40 CFR Part 158, Appendix A.



The Physical and Chemical properties for Homobrassinolide are depicted in Table 1. These data show that this compound is stable for 14 days at 54 ± 2^0 C and 62% humidity and non flammable at temperatures up to 100^0 C. The melting range is 116 - 118^0 C. The partition coefficient of log $P_{ow} = 3.96$ suggests moderate lipofilicity. Solubility in various media are as follows: in water, 3.18%, in acetone, 95.89% and in ethanol, 99.89%. The registrant should provide the MSDSs or specification sheets for the beginning materials are provided for the listed suppliers and 2) the waivers for the one-year studies of storage stability and corrosion characteristics are granted.

Table 1. Physical and Chemical Properties for Homobrassinolide Technical

Guideline Reference No./Property		Description of Result	Methods
830.6303	Color	White to pale yellow	Visual Observation
830.6303	Physical State	Powder	Visual Observation
830.6304	Odor	Mild, characteristic odor (1% suspension in ultrapure water)	Olfactory inspection
830.6313	Stability	Stable for 14 days at 54 ± 2^0 C and 62% relative humidity. The product is packaged in HDPE containers with LDPE liners, and is not likely to contact metals during its lifetime or use.	CIPAC MT 46

Guideline Reference No./Property	Description of Result	Methods
830.6314 Oxidation/Reduction Chemical Incompatibility	Not applicable, product is not intended to contact strong oxidizing or reducing agents.	
830.6315 Flammability	Not flammable at temperatures up to 100^0 C	OPPTS 6315 Closed Cup
830.6316 Explodability	Product has no potential to explode	
830.6317 Storage Stability	Stable for 14 days at 54 ± 2^0 C and 62% relative humidity. **Waiver of one-year study is requested, but is denied.**	CIPAC MT 46
830.6319 Miscibility	Not applicable, product is not to be mixed with petroleum solvents.	
830.6320 Corrosion Characteristics	Calculated corrosion rates based on mean weight loss of coupons exposed to the product for 14 days: Copper - 0.00142 mm/year Aluminum - 0.0540 mm/year. **Waiver of one-year study is requested, but is denied.**	ASTM G31-72 & D1384-87
830.6321 Dielectric Breakdown Voltage	Not required for TGAI/MP	
830.7000 pH	7.65 (1g in 100 ml of water)	CIPAC MT 75.1 Digital pH meter
830.7050 UV/Visible Absorption	^max = 1.2458 at 202 nm (10 mg in 50 ml methanol-HCL)	OECD 101
830.7100 Viscosity	Not applicable, product is a solid	
830.7220 Melting Range	116-118^0 C	OECD 102
830.7220 Boiling Range	Not applicable, product is a solid	
830.7300 Density/Relative density/Bulk Density	Density = 0.7 g/ml at ambient temperature	OECD 109 Pycnometer
830.7550 Partition Coefficient	Log P_{ow} = 3.96	OECD 117
830. 7370 Dissociation Constant in Water	pKa = 6.44 at pH 6.0 6.29 at pH 6.5 6.54 at pH 7.0 7.56 at pH 7.5 7.81 at pH 8.0	OECD 112
830.7840 Water Solubility	3.18% in water 95.89% in acetone 99.89% in ethanol	CIPAC MT 157.1 & 157.2

Tier I Toxicity Studies (MRID 47208903 – 47185106, 47185118, 47185120 – 47185124, 47185127, 47208907,)

Toxicological Profile for Homobrassinolide is presented in Table 2.0 These data suggest that the compound is practically non-toxic (Toxicity Category III and IV). This compound should not

cause chromosomal aberrations or cytotoxicity and is not a sensitizer or a dermal irritant. Exposure to eyes results in a mild irritation. Study summaries are listed by Guideline below (Table 2.0)

Table 2.0 Toxicological Profile for Homobrassinolide Technical

Study Type/OPPTS Guideline	$LD_{50}/LC_{50}/EC_{50}$ Results	Toxicity Category	MRID
Acute Oral Toxicity – Mice (OPPTS 870.1100)	> 5,000 mg/L	IV	47208903
Acute Oral Toxicity (OPPTS 870.1100)	> 5,000 mg/kg **Acceptable**	IV	47185118
Acute Dermal Toxicity (OPPTS 870.1200)	> 2,000 mg/kg **Acceptable**	III	47185120
Acute Inhalation Toxicity (OPPTS 870.1300)	>2.26 mg/L **Acceptable**	IV	47185121
Acute Eye Irritation (OPPTS 870.2400)	Mild irritation **Acceptable**	III	47185122
Acute Dermal Irritation (OPPTS 870.2500)	Not an irritant **Acceptable**	IV	47185123
Skin Sensitization (OPPTS 870.2600)	**Waiver Unacceptable, but Upgradeable**	-	47185124
90-day Oral Toxicity-rat (OPPTS 870.3100)	> 1,000 mg/L **Acceptable**	III	47208906
90-Day Dermal Toxicity (OPPTS 870.3250)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	47185136
90-Day Inhalation Toxicity (OPPTS 870.3465)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	47185137
Pre-natal Developmental (OPPTS 870.3700)	. The registrant did not provide any proof for the statement that homobrassinolide is rapidly metabolized and degraded in plant tissue.	**Waiver Request Denied**	47185139
Bacterial Reverse Mutation Assay (OPPTS 5100)	**Unacceptable.** Doses tested were too low (see summary below)	**Waiver Request Denied**	47185139
In vivo Mammalian Cytogenetics-Erythrocyte Micronucleus assay (OPPTS 870.5395)	No a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any	**Acceptable**	47185127

Study Type/OPPTS Guideline	$LD_{50}/LC_{50}/EC_{50}$ Results	Toxicity Category	MRID
	treatment dose (2000 mg/kg). **Not Cytotoxic**		
in vitro Mammalian Cell Gene Mutation Testing (OPPTS 870.5300)	No potential for genotoxicity	**Waver Request Acceptable**	**47185132**
Mammalian Bone Marrow Chromosomal Aberration Test (OPPTS 870.5385)	No chromosome aberrations in mice treated up to a single oral dose of 2000 mg/kg body weight. **No Chromosomal Aberrations**	**Acceptable**	**47208905**
DNA Synthesis in Mammalian Cells in Culture (OPPTS 870.5550)	Unscheduled DNA synthesis in mammalian cells in culture is not required	**Waiver is not needed**	**47185133**
in vitro Mammalian Chromosomal Aberration Test (OPPTS 870.5375)	The weight of evidence from studies MRID 47208905, 47185127, and the limited information from 4708904 suggest that the information submitted is sufficient to grant a waiver for this study.	**Waver Request Acceptable**	**47185134**
Immunotoxicity (OPPTS 880.3550)	No changes in organ weights (e.g., thymus, spleen) or differential white blood cell counts of the treated animals, which would indicate potential interference with normal immune function (MRID 47208906).	**Waver Request Acceptable**	**47185135**
Immune Response (OPPTS 880.3800)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	**47185140**
Chronic Exposure (OPPTS 870.4100)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	**47185141**
Carcinogenicity (OPPTS 870.4200)	A carcinogenicity test is not required	**Waiver is not needed.**	**47185142**

MRID 47208903: Acute Oral Toxicity – Mice (OPPTS 870.1100). The mouse oral LD_{50} for male, female, and combined was greater than 5000 mg/kg. **Acceptable Toxicity Category IV.**

MRID 47185120: Acute Dermal Toxicity - Rats (OPPTS 870.1200). The dermal LD_{50} for males, females, and combined was greater than 2000 mg/kg. **Acceptable: Toxicity Category III.**

MRID 4785121: Acute Inhalation Toxicity - Rats (OPPTS 870.1300). The inhalation LC_{50} for

males, females, and combined was > 2.26 mg/L. **Acceptable. Toxicity Category IV.**

MRID 47185122: Acute Eye Irritation - Rabbits (OPPTS 870.2400). Corneal opacity was noted on 6/6 rabbits at one hour after test material instillation with resolution by day 7. Iritis was noted on 6/6 rabbits 24 hours after test material instillation with resolution by day 5. Positive conjunctival irritation (score 2 or 3) was noted on 1/6, 6/6, and 6/6 rabbits 1, 24, and 48 hours after test material instillation with resolution by 72 hours. The maximum average score was 41.33 at 24 hours after test material instillation. Homobrassinolide Technical was moderately irritating. **Acceptable. Toxicity Category III.**

MRID 47185123: Primary Dermal Irritation - Rabbits (OPPTS 870.2500). No dermal irritation was noted on any rabbit. The primary irritation index was 0.0. Homobrassinolide Technical was non-irritating. **Acceptable. Toxicity Category IV.**

MRID 47185124: Skin Sensitization - Guinea Pigs (OPPTS 870.2600). After three consecutive weekly inductions, the test and control animals showed no signs of reactivity at 24 and 48 hours after challenge. The study did not include a positive control study which was carried out within six months of the study. Homobrassinolide Technical would not be a dermal sensitizer if the registrant provides a positive control study carried out within six months of the study and the results are appropriate. **Unacceptable, but upgradeable** if the registrant provides a positive control study which was carried out within six months of the study and the results are appropriate.

MRID 47185131: Waiver Request for Hypersensitivity Incidents. No incidents have been reported regarding sensitivity to Homobrassinolide. These reports are submitted as 6(a) 2.

MRID 47208906 : 90-Day Oral Toxicity Gavage - Rat; (OPPTS 870.3100). This 90-day oral toxicity study with recovery in the Wistar rat is upgraded to **Acceptable:** The NOAEL for Homobrassinolide Technical was 1000 mg/kg/day. There were no changes in organ weights (e.g., thymus, spleen) or differential white blood cell counts of the treated animals, which would indicate potential interference with normal immune function.

MRID 47185136: Waiver Request for 90-Day Dermal Toxicity (OPPTS 870.3250). Since the 90-day oral toxicity study was found to be acceptable the information submitted is sufficient to support the requested waiver for 90-day dermal testing. The active ingredient should not present any purposeful application or prolonged exposure to human skin and is not expected to be metabolized differently after dermal exposure. This waiver is **Acceptable**.

MRID 47185137: Waiver Request for 90-Day Inhalation Toxicity (OPPTS 870.3465). The ORNL reviewer for the 90-day oral toxicity study found it to be **Acceptable**. Based on the expected use patterns, there is no likelihood of significant inhalation levels from exposure to the pesticide as a gas, vapor, or aerosol. Therefore, the information submitted is sufficient to support the requested waiver for 90-day inhalation testing.

MRID 47185138: Waiver Request for Prenatal Developmental - Rat (OPPTS 870.3700). The registrant did not provide any proof for the statement that homobrassinolide is rapidly metabolized and degraded in plant tissue. The prenatal developmental-rat test is a requirement for registration. The bacterial mutation assay **(MRID 47208904)** cited above is a genotoxicity study and cannot be used to fulfill OPPTS 870.3700 requirements. Therefore, the information submitted is not sufficient to support the requested waiver for teratogenicity. **Waiver denied.**

MRID 47185139: Waiver Request for Prenatal Developmental OPPTS 870.3700). This is a Tier I requirement because this product has the potential for widespread use that can result in exposure to females. Therefore, a **waiver is denied for this compound.**

MRID 47208904: Bacterial Reverse Mutation Test; (Bacterial system, *Salmonella typhimurium*)/ mammalian activation gene mutation assay; OPPTS 870.5100.Homobrassinolide Technical at dose levels of 0.03 to 0.05 µg/plate was non-mutagenic in *Salmonella typhimurium* strains TA1537, TA1535, TA98, and TA100. **Unacceptable.** The doses that were tested were extremely low: the highest dose was 10^4 times lower than the limit dose of 5,000 µg/plate recommended by the Guidelines. There was no mention of toxicity caused by the test substance, with or without activation in any tester strain. However, the study is deficient and unacceptable because the researchers used a range of low doses (0.03 to 0.5 µg/plate) ignoring higher dose ranges.. In addition, only 4 of the 5 recommended strains were tested. Almost no information was provided on the S9 fraction used for activation. The number of bacteria plated for each treatment group was not presented, making it impossible to quantitate the number of revertants/10^6 viable bacterial cells. No historical control data were presented.

MRID 47185132: Waiver Request for *in vitro* Mammalian Cell Gene Mutation Testing (OPPTS 870.5300). The Bacterial Reverse Mutation Tests (47208904) was deemed unacceptable because of insufficient dosage levels. However, at the concentrations tested, there were not mutation issues. Therefore, considering the results from studies MRID 47208905 and 47185127 in addition to the limited information from MRID 4708904, the Agency has considered that this weight of evidence is sufficient to grant a waiver for the *in vitro* mammalian cell gene mutation testing. **Waiver is Acceptable.**

MRID 47185134: Waiver Request for in vitro Mammalian Chromosomal Aberration Test (OPPTS 870.5375). The information submitted is sufficient to support the requested waiver an *in vitro* mammalian chromosomal aberration test. **This waiver is Acceptable**

MRID 47208905. *In Vivo* Mammalian Cytogenetics - Erythrocyte chromosomal aberration assay in mice (OPPTS 870.5385). In a chromosomal aberration test, Homobrassinolide Technical did not have the potential to induce chromosome aberrations in mice treated up to a single oral dose of 2,000 mg/kg body weight. **This study is Acceptable** and in general satisfies the guideline requirement for Test Guideline (OPPTS 870.5385) for *in vivo* cytogenetic mutagenicity data. NOTE: This is a Tier II study.

MRID 47185127: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus assay in mouse OPPTS 870.5395. In this micronucleus test Homobrassinolide Technical did not have micronucleus induction potential in mice after two days of oral dosing up to a level of 2,000 mg/kg body weight. **This study is Acceptable** and satisfies the guideline requirement. NOTE: This is a Tier II study.

MRID 47185133: Waiver Request for Unscheduled DNA Synthesis in Mammalian Cells in Culture (OPPTS 870.5550). A test for unscheduled DNA synthesis in mammalian cells in culture is not required, and a **waiver is therefore not needed because this study isn't in any of the Agencies current Tiers studies.**

MRID 47185135: Waiver Request for Immunotoxicity (OPPTS 880.3550). In a 90-day oral toxicity study in rats (MRID 47208906), the NOAEL for Homobrassinolide Technical was 1,000 mg/kg/day. There were no changes in organ weights (e.g., thymus, spleen) or differential white blood cell counts of the treated animals, which would indicate potential interference with normal immune function. This submitted information **is sufficient to support the requested waiver. This waiver is Acceptable.**

MRID 47185140: Waiver Request for Immune Response (OPPTS 880.3800).). **This is a Tier III study and is not required at this stage of registration.**

MRID 47185141: Waiver Request for Chronic Exposure (OPPTS 870.4100). **This is a Tier III study and is not required at this stage of registration.**

MRID 47185142: Waiver Request for Carcinogenicity (OPPTS 870.4200). **This is a Tier III study and is not required at this stage of registration.**

Tier I Non-Target Organism Studies and Waiver Requests

Table 3.0 Toxicological Profile for Homobrassinolide Technical

Study Type/OPPTS Guideline	$LD_{50}/LC_{50}/EC_{50}$ Results	Toxicity Category	MRID
Avian Acute Oral Toxicity Test (OPPTS 850.2100)	Waiver based on low acute exposure & low toxicity (see summary below for details) **Acceptable**	**Practically non-toxic based on waiver information**	**47185129**
Avian Dietary Toxicity Test (OPPTS 850.2200)	Waiver based on regular exposure to naturally-occurring substance in nature & low toxicity (see summary below for details) **Acceptable**	**Practically non-toxic based on waiver information**	**47185143**
Fish Acute Toxicity Test, Freshwater and Marine	96 Hr LC_{50} =24.56 mg/L (20.44-28.68 mg/L)	**Slightly Toxic**	**47185129**

Study Type/OPPTS Guideline	$LD_{50}/LC_{50}/EC_{50}$ Results	Toxicity Category	MRID
(OPPTS 850.1075)	**Acceptable** 96 hr LC_{50} =14.38 mg/L (12.79-15.98 mg/L) **Acceptable**		
Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (OPPTS 850.1010)	48-hr EC_{50} = 8.90 mg/L (8.47 to 9.34 mg/L. **Acceptable**	**Moderate Toxicity**	47185130
Non-Target Plants (OPPTS 850.4000)	Waiver based on natural occurrence of substance in plants and low application rate	**Practically non-toxic based on waiver information**	47185145
Non-Target Insects (OPPTS 850.	Waiver based on natural occurrence of substance in plants, lack of known insect toxicity, and regular exposure to insects via plant contact.	**Practically non-toxic based on waiver information**	47185146

Ecotoxicity data shows that homobrassinolide is slightly toxic to freshwater fish (14.38 – 24.56 mg/L) and moderately toxic to aquatic invertebrates (8.90 mg/L) (see Table 3).

MRID 47185129: Homobrassinolide Technical. Biochemical Pesticides Nontarget Organisms Toxicology Data. Acute Immobilization Test to Freshwater Fish, *Poecilia reticulata* and *Brachydanio rerio*. 96 Hr LC_{50} 24.56 mg/L (20.44-28.68 mg/L) 96 hr LC_{50}=14.38 mg/L (12.79-15.98 mg/L). This study was conducted as a static renewal and is **Acceptable**.

MRID: 47185130: Homobrassinolide Technical. Biochemical Pesticides Nontarget Organisms Toxicology Data. Acute Immobilization Test in *Daphnia magna*. 48-hr EC_{50} = 8.90 mg/L (8.47 to 9.34 mg/L. This study was conducted as a static renewal and is **Acceptable.**

MRID 47185143: Waiver Request for Avian Acute Oral Toxicity (OPPTS 850.2100). The registrant calculated the anticipated application rate of homobrassinolide as follows: 20 g/A = 20,000 mg/43,560 ft^2 = 0.459 mg/ft^2. Homobrassiolide do not appear to be toxic to vertebrates. Toxicity studies on mammals show practically no toxicity (i.e. rat LD_{50} > 5,000 mg/kg; mice LD_{50} > 5, 000 mg/kg; 90-day rat oral was 1,000 mg/kg). In addition, avian species have been environmentally exposed to the active ingredient since Homobrassinolides are found in all plants. The information submitted is sufficient to support the requested waiver for Avian Acute Oral Toxicity testing. **This waiver is Acceptable.**

MRID 47185144: **Waiver Request** for Avian Acute Dietary Toxicity (OPPTS 850.2200) The information submitted is sufficient to support the requested waiver for Avian Acute Dietary Toxicity testing. Homobrassiolide do not appear to be toxic to vertebrates. Toxicity studies on

mammals show practically no toxicity (i.e. rat LD_{50} > 5,000 mg/kg; mice LD_{50} > 5, 000 mg/kg; 90-day rat oral was 1,000 mg/kg). In addition, avian species have been environmentally exposed to the active ingredient since Homobrassinolides are found in all plants. Application of the product in the environment is expected to be low at 20 g/A with potential exposure to avian species at less than 1 mg/ft^2. The compound is to be sprayed and not applied as a granular, thus decreasing exposure. **This waiver request is Acceptable.**

MRID 47185145: Waiver Request for Nontarget Plant Studies (OPPTS 850.4000). Since, homobrassinolide occurs naturally in plants, it is unlikely that exogenous application can cause any adverse effects to nontarget plants. In addition, there does not appear to be any reported incidents of toxicity to plants. An expected application rate (20 g/A (1 mg ft^2)) of a Homobrassinolide end use product appears to be very low. Therefore, the information submitted is sufficient to support the requested waiver for nontarget plant studies. This waiver request is **Acceptable.**

MRID 47185146: Waiver Request for Nontarget Insect Testing (OPPTS 850.4350). This compound does not exhibit any insecticidal activity. Since Homobrassinosteroids and brassinolide are assumed to be ubiquitous in all plants and plant products (i.e. pollen) insects have always been exposed to these compounds without displaying toxic effects. The information submitted is sufficient to support the requested waiver for nontarget insect testing. **This waiver is Acceptable.**

Risk Assessment

Brassinosteroids are a group of steroidal plant hormones that were discovered in 1973, when it was shown that pollen from *Barssica napus* could promote stem elongation and cell divisions and that the biologically active molecule was a steroid (Seeta *et al.*, 2002). Since their discovery, over 70 brassinosteroids have been isolated from plants. The occurrence of these steroids have been demonstrated in various plant parts, such as pollen, flower buds, fruits seeds, vascular cambium, leaves, shoots and roots. Studies on higher plants suggest that these steroids play a critical role in a range of developmental processes (i.e. stem elongation, root growth, floral initiation, etc). The molecular mode of action of brassinosteroids is still being studied. However, the binding of the brassinosteroid molecule to a receptor in the cell plasma membrane appears to activate the kinase domain and subsequent phosphorylation of additional kinases and/or phosphotases (Seeta *et al.* 2002). The active involvement of brassinosteroids in various aspects of plant growth and development is regulated by their action on proton pump, resulting in cell elongation through an increase in cell wall elasticity (Bajguz, 2000) and the orientation of the microtubules (Catterou *et al.*, 2000).

Homobrassinolide Technical is an active ingredient/manufacturing use product to be used only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% w/w homobrassinolide (2α, 3α, 22S, 3S, 24S)-2, 3, 22, 23-tetrahydroxy-24-ethyl-β-homo-7-oxa-5α-

cholestan-6-one). There are no intentionally-added inert ingredients in the product. Impurities in the product are [redacted] The CSF and product label are in agreement regarding the content of active ingredient. The beginning materials were described, but the MSDSs submitted were not from the suppliers specified in MRID 47185101. There was information showing that the compound is produced using an integrated process, as well as an adequate discussion of the formation of the impurities. Acceptable results from analysis of five lots of Homobrassinolide Technical were submitted. The certified limits for the active ingredient are within the OPPTS 830.1750 guidelines. The enforcement analytical method is high performance liquid chromatography with ultraviolet detection. The physical/chemical characteristics were adequately presented. Results of acute studies with Homobrassinolide Technical are summarized in Table 2. Based on those studies, the registrant found the test material to be practically non-toxic, with no human/mammalian health problems expected (Toxicity Category III and IV).

Human Health

Toxicological Profile for Homobrassinolide is presented in Table 3. This data suggests that the compound is in Toxicity Category III – IV for oral and dermal toxicity, Toxicity Category IV for acute inhalation toxicity, Toxicity Category III for primary eye irritation (exposure to eyes results in a mild irritation), and Toxicity Category IV for primary dermal irritation (not a dermal sensitizer). Studies also show that homobrassinolide is not cytotoxic and that it should not cause chromosomal aberrations. Prenatal Developmental effects have not been evaluated and the request for a waiver was denied because this is a Tier I requirement and the registrant did not provide proof that the active ingredient is rapidly metabolized and degraded in plant tissue. The request for a waiver for immunotoxicity was deemed acceptable because the 90-day oral toxicity study in rats (MRID 47208906) showed a NOAEL of > 1000 mg/kg/day with no changes in organ weights or differential white blood cell counts. This suggests the homobrassinolide should not cause potential interference with normal immune function.

Dietary Exposure to Humans

The registrant has provided information on the safety of homobrassinolide which covers human and environmental safety. Although the metabolism of brassinosteroids is poorly understood, these plant steroids appear to be metabolized in plants to give the inactive forms, through transformation in the side chain or in the steroid skeleton. Brassinosteroids are present in all plants, but appear to result in no harm to humans and other organisms even though there is ubiquitous exposure via the food chain. The endogenous levels are in ppm to ppb levels (i.e. brassinosteroids levels in pollen have measured at 200 ppb). The registrant claims that the small quantity (< 20 g/A) of Homobrassinosteroids that is proposed for application as a plant growth stimulant is unlikely to increase levels of brassininosteroids in the treated plants. They reason that the amount of exogenenous brassinosteroid that is applied to a crop plant will be

metabolized as the plant grows. Therefore, levels in the human diet are unlikely to be affected. Calculations suggests that human/wildlife exposure to Homobrassinosteroids is expected to be low with 20 g/A = 20,000 mg/43,560 ft^2 = 0.459 mg/ft^2. Potential toxicity is low with acute oral toxicity value > 5 g/kg/bw, acute dermal > 2 g/kg/bw, and acute inhalation is 2.2 mg/L. This suggests that risk from exposure is very low at 20 g/A and that even if an entire acre of exposed plants were consumed (unlikely) by an individual, the toxicity from Homobrassinosteroid is >10X below the toxicity values noted in Table 3. The applicant has requested tolerance exemption for residues of homobrassinolide in or on crops and use sites. The registrant has also requested waivers for tests used to determine residues, as well as the analytical method that should be used. Based on the available data, the Agency feels that there are reasonable grounds (as noted above) for tolerance exemption for the proposed uses of homobrassinolide in/on all raw agricultural commodities. The Agency also accepts the registrant's argument that for low homobrassinolide toxicity and exposure to humans/wildlife and feels that exemption from the required tolerance of residues in or on raw commodities is warranted.

Occupational and Residential Exposure

The product is not intended for homeowner (residential) use and therefore, there will be no residential exposure. The label statement is for manufacturing use only. Directions for use state that the ...product is intended only for formulation into plant growth regulator end-use products. Each formulator is responsible for obtaining EPA registration of the respective end-use product(s). Although this compound is expected to be used on all crops there are no labels for specific crops.

Drinking Water

It seems unlikely that homobrassinolide concentrations would exceed levels that are currently ubiquitous to plants. Although Fate information is not available, the compound is not soluble in water (water solubility 3.18%), and the log P_{ow} = 3.96 suggests moderate binding to soil and a low probability of ground water contamination.

Non-Target Organism Hazard Assessment and Endangered Species Concerns

The proposed uses of homobrassinolide include mostly pre-harvest applications on agricultural crops, and ornamental and forest trees (terrestrial, food crops, greenhouse food and non-food crops, aquatic food and non-food crops). The uses cover all crops (food and non-food), grasses, vines and trees listed under 40CFR Part 158, Appendix A. Ecotoxicity data shows that this compound is slightly toxic to freshwater fish (LC_{50} = 14.38 – 24.56 mg/L) and moderately toxic to aquatic invertebrates (EC_{50} = 8.90 mg/L). Toxicity to mammals is in the slight to practically non-toxic range (LD_{50} >1,000 to 5,000 mg/kg). Since this compound is ubiquitous to plants, and has low toxicity to vertebrates and invertebrates, it can be assumed that there is low to no toxicity to other non-target organisms such as plants, avian, and insects. In evaluating possible impact to aquatic and terrestrial endangered species the Agency extends the above assumptions and data and predicts no direct or indirect impact to endangered species from the use of homobrassinolide.

This was determined by employing the Individual Chance Model version 1.1 that uses the probit dose-response curve and the median lethal estimate in predicting effects to an individual. The default slope of 4.5 was used in determining the chance of an individual effect. Using the acute endangered species level of concern (LOC) of 0.05 and the default slope of 4.5 the chance of an individual mortality for aquatic endangered species is ~ 1 in 410,000,000 and for terrestrial endangered species it is ~ 1 in 294,000.

Outstanding Data Requirements

The outstanding data requirements that must be submitted prior to registration of Homobrassinolide are as follows:

1) **Bacterial Reverse Mutation Tests (870.5100):** Since the registrant did not provide the necessary dosage range, a new study is required prior to registration.

2) **Skin Sensitization - Guinea Pigs (870.2600):** The study did not include a positive control study which was carried out within six months of the study. The study was unacceptable but upgradeable if the information is submitted prior to registration.

3) **Storage Stability (830.6317):** This study is required under 158.930 of the Federal Register (Vol. 71, No. 45/Wednesday, March 8, 2006).

4) **Corrosion (830.6320):** This study is required under 158.930 of the Federal Register (Vol. 71, No. 45/Wednesday, March 8, 2006).

5) **Prenatal Developmental-Rat (OPPTS 870.3700):** This is a Tier I requirement. The registrant did not provide any proof for the statement that homobrassinolide is rapidly metabolized and degraded in plant tissue.

References

Bajguz, A., 2000. Effect of brassinosteroids on nuclear acids and protein content in cultured cells of *chlorella vulgaris. Plant Physiol. Biochem.* **38, 209-215.**

Catterou, M., F. Dubois, H. Schaller, L. Aubanella, B. Vilcol, B. S. Sangwan-Norrel, R.S. Sangwan, 2001. Brassinosteroids microtubules and cell elongation in Arabidopsis thaliana. II. Effects of brassinosteroids on microtubules and cell elongation in the bull mutant. *Planta,* **212, 673-683.**

Seeta S.R.R., B.V.Vardhini, E. Sujatha, S. Anuradha. 2002. Brassinosteroids-A new class of phytohormones. *Current Science,* **82:12391245**.

cc: *Reviewer name Miachel Rexrode, RAL name*, BPPD Chron File, IHAD/ARS
Reviewer name, FT, PY-S: *date*

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: **March 27, 2009**

SUBJECT: Science review of resubmitted studies (MRID 47546501-03) in support of a Tolerance Exemption Petition, registration of an End-Use Product and Request for Waivers on Certain Data Requirements for Homobrassinolid and the Registration of **Homobrassinolide Technical**

Decision Number:	381556
DP Number:	357249
EPA File Symbol Number:	69361-RT
Chemical Class:	Biochemical
PC Code:	067700
CAS Number:	80483-89-2
Tolerance Exemptions:	Pending
MRID Numbers:	47546501-03

FROM: Miachel Rexrode Ph.D., Senior Biologist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

TO: John Fournier, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

THE FOLLOWING CONTAINS CONFIDENTIAL BUSINESS INFORMATION

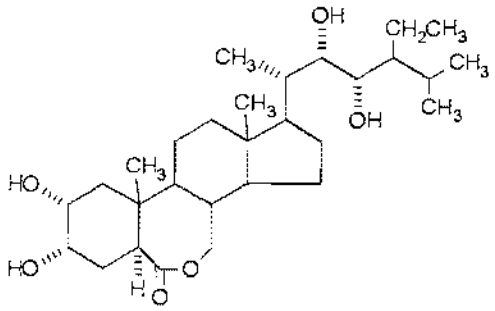
ACTION REQUESTED

1) Repar Corporation (Repar) has **resubmitted studies** in support of a tolerance exemption petition for the active ingredient, homobrassinolide(2α,3α,22S,23S,24S)-2,3,22,23-tetrahydroxy-24-ethyl-β-homo-7-oxa-5α-cholestan-6-one), on all raw agricultural commodities (food and non-food crops including forage crops and animal feed as well as residues of homobrassinolide in meat, milk, and eggs). In addition to tolerance exemptions, the registrant is also requesting waivers for all residue chemistry, and all applicable data requirements for pre- and post- harvest uses on all crops (waiver requests for all applicable guidelines Series 171).

The registrant has also submitted data in support of the registration of the product Homobrassinolide Technical. In support of the Tolerance Exemption petition and registration, the registrant has submitted product chemistry studies, Tier I toxicity studies and waiver requests, and non-target organism studies and waiver requests.

Background

Brassinosteroids are a class of plant polyhydroxysteroids that are ubiquitously distributed in the plant kingdom. These compounds, when applied to plants, improve their quality and yield and have been further researched for stress-protective properties (i.e. cold, heat, salt, and heavy metal exposure). Homobrassinolide is a plant growth regulator that is proposed as controlling or regulating the growth and development of all higher and lower plants. The proposed uses include all agronomic and horticultural crops such as agricultural crops, greenhouse food uses and non-food crops, aquatic food and non-food crops (all crops, grasses, vines, and trees listed under 40 CFR Part 158, Appendix A).



RECOMMENDATIONS AND CONCLUSIONS

1a. The following studies were resubmitted for review: Dermal Sensitization (OPPTS 870.2600); Pre-Natal Developmental (OPPTS 870.3700); and Bacterial Reverse Mutation Assay (OPPTS 870.5100).

1b. MRID 47546502: Bacterial Reverse Mutation Test; (Bacterial system, *Salmonella typhimurium*)/ mammalian activation gene mutation assay; OPPTS 870.5100. **Acceptable.**

1c. MRID 475446501: Resubmission Skin Sensitization - Guinea Pigs (OPPTS 870.2600). **Acceptable**

1d. MRID 47185139: Resubmission Waiver Request for Prenatal Developmental - Rat OPPTS 870.3700). **Unacceptable**

STUDY SUMMARIES

MRID 47208904: Previous Study Bacterial Reverse Mutation Test; (Bacterial system, *Salmonella typhimurium*)/ mammalian activation gene mutation assay; OPPTS 870.5100. **Unacceptable.** The doses that were tested were extremely low: the highest dose was 10^4 times lower than the limit dose of 5,000 µg/plate recommended by the Guidelines. There was no mention of toxicity caused by the test substance, with or without activation in any tester strain. However, the study is deficient and unacceptable because the researchers used a range of low doses (0.03 to 0.5 µg/plate) ignoring higher dose ranges.. In addition, only 4 of the 5 recommended strains were tested. Almost no information was provided on the S9 fraction used for activation. The number of bacteria plated for each treatment group was not presented, making it impossible to quantify the number of revertants/10^6 viable bacterial cells. No historical control data were presented.

MRID 47546502: Resubmission Bacterial Reverse Mutation Test; (Bacterial system, *Salmonella typhimurium*)/ mammalian activation gene mutation assay; OPPTS 870.5100. After range finding testing with technical homobrassinolide (87.1% ai), researchers chose five dosage levels ranging from 39.06 - 625 ug/plate for strains TA100, TA1535, TA98, and 9.77 - 156.25 ug/plate for strains TA102 and TA1537 with and without S9. Testing appeared to have been conducted according to standard protocol. Results show that there was no concentration related or reproducible increase in the number of revertant colonies in the concentrations tested and no observable statistically significant dose-response relationship. The test substance, homobrassinolide, did not induce any apparent mutagenic effect in the *Salmonella typhimurium* strains tested. This resubmitted test is considered **Acceptable.**

MRID 47185124: Previous Study Skin Sensitization - Guinea Pigs (OPPTS 870.2600). **Unacceptable, but upgradeable** if the registrant provides a positive control study which was carried out within six months of the study and the results are appropriate.

MRID 475446501: Resubmission Skin Sensitization - Guinea Pigs (OPPTS 870.2600). None of the animals of treatment groups and control groups presented any skin irritation at 24 and 48 hour after removal of the challenge patch. This study is **Acceptable**.

MRID 47185138: Previous Waiver Request for Prenatal Developmental - Rat (OPPTS 870.3700). **Waiver denied.**

MRID 47185139: Resubmission Waiver Request for Prenatal Developmental - Rat OPPTS 870.3700). This is a Tier I requirement because this product has the potential for widespread use that can result in exposure to females. This compound is a phytosteroid with structure similar to B-sitosterol. Although the registrant provided literature information that the compound is metabolized and degraded in plant tissue, this was never quantitated and the registrant did not supply any information regarding metabolism in animals. Therefore, the prenatal developmental-rat test is a requirement for registration. The bacterial mutation assay **(MRID 47208904)** cited above is a genotoxicity study and cannot be used to fulfill OPPTS 870.3700 requirements. Therefore, the information submitted is not sufficient to support the requested waiver for teratogenicity. **Waiver denied.**

Tier I Toxicity Studies (MRID 47208903 – 47185106, 47185118, 47185120 – 47185124, 47185127, 47208907,)

Toxicological Profile for Homobrassinolide is presented in Table 1.0 These data suggest that the compound is practically non-toxic (Toxicity Category III and IV). This compound should not cause chromosomal aberrations or cytotoxicity and is not a sensitizer or a dermal irritant. Exposure to eyes results in a mild irritation. Study summaries are listed by Guideline below (Table 1.0)

Table1.0 Toxicological Profile for Homobrassinolide Technical

Study Type/OPPTS Guideline	$LD_{50}/LC_{50}/EC_{50}$ Results	Toxicity Category	MRID
Acute Oral Toxicity – Mice (OPPTS 870.1100)	>5,000 mg/L **Acceptable**	IV	47208903
Acute Oral Toxicity (OPPTS 870.1100)	> 5,000 mg/kg **Acceptable**	IV	47185118
Acute Dermal Toxicity (OPPTS 870.1200)	> 2,000 mg/kg **Acceptable**	III	47185120

Study Type/OPPTS Guideline	$LD_{50}/LC_{50}/EC_{50}$ Results	Toxicity Category	MRID
Acute Inhalation Toxicity (OPPTS 870.1300)	>2.26 mg/L **Acceptable**	**IV**	**47185121**
Acute Eye Irritation (OPPTS 870.2400)	Mild irritation **Acceptable**	**III**	**47185122**
Acute Dermal Irritation (OPPTS 870.2500)	Not an irritant **Acceptable**	**IV**	**47185123**
Skin Sensitization (OPPTS 870.2600)	No skin irritation at 48 hrs **Acceptable**	**IV**	**475446501**
90-day Oral Toxicity-rat (OPPTS 870.3100)	> 1,000 mg/L **Acceptable**	**III**	**47208906**
90-Day Dermal Toxicity (OPPTS 870.3250)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	**47185136**
90-Day Inhalation Toxicity (OPPTS 870.3465)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	**47185137**
Pre-natal Developmental (OPPTS 870.3700)	The registrant did not provide any proof for the statement that homobrassinolide is rapidly metabolized and degraded in plant tissue.	**Waiver Request Denied**	**47185139**
Bacterial Reverse Mutation Assay (OPPTS 5100)	Five dosage levels ranging from 39.06 - 625 ug/plate for strains TA100, TA1535, TA98, and 9.77 - 156.25 ug/plate for strains TA102 and TA1537 with and without S9. Testing showed that there was no concentration related or reproducible increase in the number of revertant colonies in the concentrations tested and no observable statistically significant dose-response relationship. This resubmitted test is considered **Acceptable.**	No mutagenic effect in the *Salmonella typhimurium* strains tested.	**47546502**
In vivo Mammalian Cytogenetics-Erythrocyte Micronucleus assay (OPPTS 870.5395)	No a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment dose (2000 mg/kg). **Acceptable**	**Not Cytotoxic**	**47185127**

Study Type/OPPTS Guideline	$LD_{50}/LC_{50}/EC_{50}$ Results	Toxicity Category	MRID
in vitro Mammalian Cell Gene Mutation Testing (OPPTS 870.5300)	No potential for genotoxicity	**Waver Request Acceptable**	**47185132**
Mammalian Bone Marrow Chromosomal Aberration Test (OPPTS 870.5385)	No chromosome aberrations in mice treated up to a single oral dose of 2000 mg/kg body weight. **Acceptable**	**No Chromosomal Aberrations**	**47208905**
DNA Synthesis in Mammalian Cells in Culture (OPPTS 870.5550)	Unscheduled DNA synthesis in mammalian cells in culture is not required	**Waiver is not needed**	**47185133**
in vitro Mammalian Chromosomal Aberration Test (OPPTS 870.5375)	The weight of evidence from studies MRID 47208905, 47185127, and the limited information from 4708904 suggest that the information submitted is sufficient to grant a waiver for this study.	**Waver Request Acceptable**	**47185134**
Immunotoxicity (OPPTS 880.3550)	No changes in organ weights (e.g., thymus, spleen) or differential white blood cell counts of the treated animals, which would indicate potential interference with normal immune function (MRID 47208906).	**Waver Request Acceptable**	**47185135**
Immune Response (OPPTS 880.3800)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	**47185140**
Chronic Exposure (OPPTS 870.4100)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	**47185141**
Carcinogenicity (OPPTS 870.4200)	A carcinogenicity test is not required	**Waiver is not needed.**	**47185142**

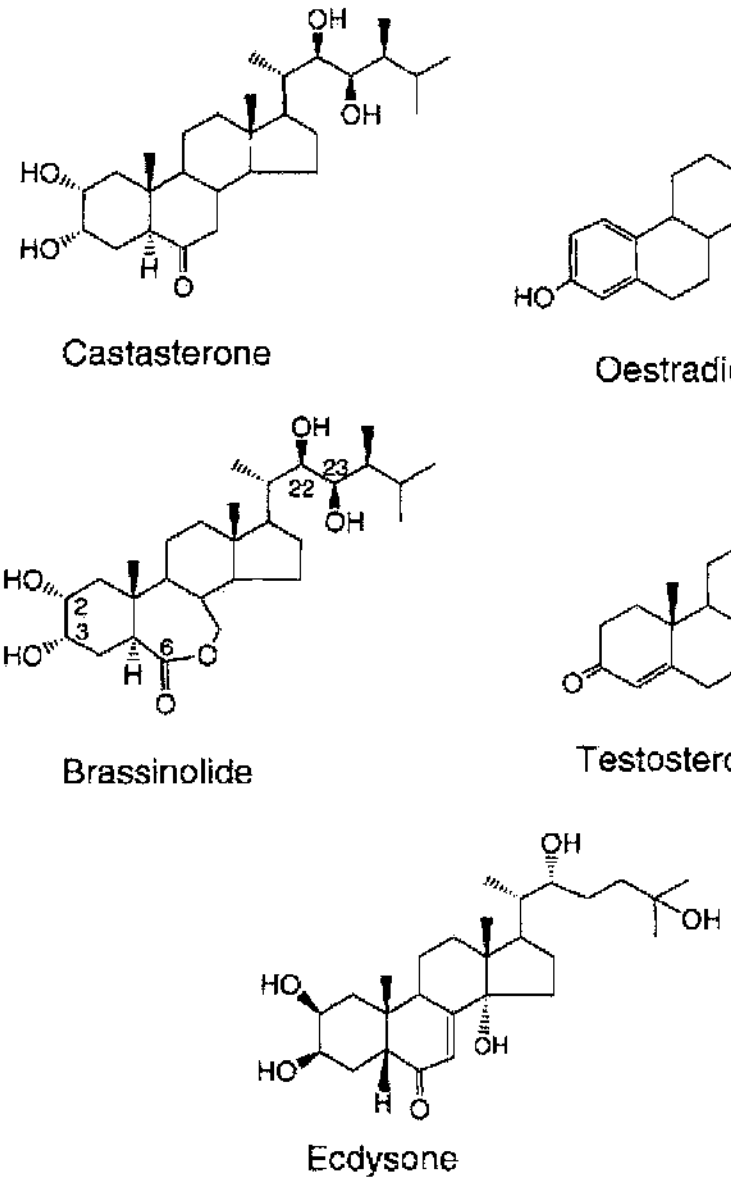


Figure 1.

Structures of Steroid Hormones.

Chemical structure of brassinolide and castasterone plant steroid hormones, in comparison with the mammalian sex steroid hormones testosterone and oestradiol, and the insect steroid hormone ecdysone. Highlighted are carbon numbers of BL having oxygen moieties that are important for BR activity.

Plant Cell. 2002; 14(Supplement): s97–s110.
doi: 10.1105/tpc.001461.

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for Nontarget Insect Testing (OPP 154-11)

MRID 47185146

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: Anthony Q. Armstrong
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for Nontarget Insect Testing (OPP 154-11)
MRID NO:	47185144
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-ECOTOX-56
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. Nontarget Insect Testing.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	The information submitted is sufficient to support the requested waiver for nontarget insect testing.

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for Nontarget Insect Testing (OPP 154-11).

Registrant's Justification

Homobrassinolide is a plant-growth regulating steroid, and has not been reported to exhibit any insecticidal activity. Literature reports on the homobrassinolide isomer epibrassinolide do not indicate severe toxicity or any kind of environmental hazard to insects or other nontarget organisms.

Furthermore, insects are already exposed to homobrassinolide and other brassinosteroids that occur naturally in extremely small amounts in all parts of plants, including flowers, pollen, fruits, and seeds. Exogenous application of homobrassinolide via the use of end use products is unlikely to produce adverse effects on nontarget insects since the anticipated application rate is very low (20 g/A, equivalent to slightly less than 1 mg homobrassinolide/ft^2).

Reviewer's Conclusion

The information submitted is sufficient to support the requested waiver for nontarget insect testing. The registrant should probably cite OPPTS 880.4350 and 850.3040 as guidelines for nontarget insect testing. The registrant calculated the anticipated application rate of homobrassinolide as follows: 20 g/A = 20,000 mg/43,560 ft^2 = 0.918 mg/ft^2. The reviewer calculates the application rate to be even lower, 0.459 mg/ft^2 . The reviewer notes that the registrant's definition of nontarget insects includes "honeybees, earthworms, dipterans and arthropods, predators and parasites."

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for Unscheduled DNA Synthesis in Mammalian Cells in Culture (OPPTS 870.5550)

MRID 47185133

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T.

Signature:
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for Unscheduled DNA Synthesis in Mammalian Cells in Culture (OPPTS 870.5550)
MRID NO:	47185133
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-TOX-43
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. Unscheduled DNA Synthesis in Mammalian Cells in Culture.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	A test for unscheduled DNA synthesis in mammalian cells in culture is not required, and a waiver is therefore not needed.

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for Unscheduled DNA Synthesis in Mammalian Cells in Culture (OPPTS 870.5550).

Registrant's Justification

In a reverse mutation study (MRID 47208904), Homobrassinolide Technical at dose levels of 0.03 to 0.05 µg/plate was non-mutagenic in *Salmonella typhimurium* strains TA1537, TA1535, TA98, and TA100.

In a chromosomal aberration test (MRID 47208905), Homobrassinolide Technical did not have the potential to induce chromosome aberrations in mice treated up to a single oral dose of 2000 mg/kg body weight.

In a micronucleus test (MRID 47185127), Homobrassinolide Technical did not have micronucleus induction potential in mice after two days of oral dosing up to a level of 2000 mg/kg body weight.

Based on the results of these three studies, the registrant concluded that Homobrassinolide Technical has no potential for genotoxicity.

Reviewer's Conclusion

The reviewer notes that the ORNL reviewer for the reverse mutation study cited above found it to be unacceptable. Regardless, ORNL does not find that an unscheduled DNA synthesis in mammalian cells in culture test would be required, and a waiver request is therefore not needed.

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for 90-Day Dermal Toxicity (OPPTS 870.3250)

MRID 47185136

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T.

Signature: Sylvia Milanez
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for 90-Day Dermal Toxicity (OPPTS 870.3250)
MRID NO:	47185136
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-TOX-46
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. 90-Day Dermal Toxicity.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	The information submitted is not sufficient to support the requested waiver for 90-day dermal toxicity testing.

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for 90-Day Dermal Testing (OPPTS 870.3250).

Registrant's Justification

In an acute dermal toxicity study in rats (MRID 47185120), the LD_{50} for Homobrassinolide Technical was >2000 mg/kg (Toxicity Category IV). In an acute dermal irritation study in rabbits (MRID 47185123), Homobrassinolide Technical was not an irritant (Toxicity Category IV).

In a 90-day oral toxicity study in rats (MRID 47208906), the NOAEL for Homobrassinolide Technical was 1000 mg/kg/day. The metabolism of Homobrassinolide Technical applied dermally is not expected to be different from that resulting from ingestion. It is therefore unlikely that dermal application will result in toxic metabolites.

Reviewer's Conclusion

The ORNL reviewer for the 90-day oral toxicity study found it to be unacceptable. Based on that conclusion, the information submitted is not sufficient to support the requested waiver for 90-day dermal testing. If the Agency judges the 90-day oral toxicity study to be acceptable, then sufficient information has been submitted to support the waiver for 90-day dermal testing.

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (OPPTS 850.1010)

MRID 47185130

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: Anthony Q. Armstrong
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (OPPTS 850.1010)
MRID NO:	47185130
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	9510
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	International Institute of Biotechnology and Toxicology (IIBAT), Padappai-601 301, Kancheepuran District, Tamil Nadu, India
TITLE OF REPORT:	Homobrassinolide Technical. Biochemical Pesticides Nontarget Organisms Toxicology Data. Acute Immobilisation Test in *Daphnia magna*.
AUTHOR:	Chittabubu, R.A.
STUDY COMPLETED:	May 9, 2006
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study was conducted in accordance with 40 CFR Part 1060.
STUDY SUMMARY:	In a 48-hour static acute toxicity test, *Daphnia magna* were exposed to test solutions containing nominal concentrations of 0, 5, 6.5, 8.5, 11.0, or 14.3 mg/L of Homobrassinolide Technical (a.i., 80.2% homobrassinolide). Percent immobility in the 0, 5, 6.5, 8.5, 11.0, and 14.3 mg/L test material groups was 0, 0, 20, 40, 70, and 100%, respectively, at the end of the test. Based on the nominal concentrations, the 48-hr EC_{50} value was 8.90 mg/L, with a 95% confidence interval of 8.47 to 9.34 mg/L.

Unacceptable. The actual concentration of

CLASSIFICATION: Homobrassinolide Technical in the test solutions was not determined.

Test Material

Homobrassinolide Technical, reported purity 80.2%, Lot No. not provided, a cream colored powder obtained from the study sponsor with an expiration date of September 23, 2006. A certificate of analysis is provided on p. 25 of MRID 47185130. After receipt at the testing facility, the test material was stored in the utility room.

Test Methods

A 48-hour static acute toxicity test was conducted to determine the acute toxicity of the test material to *Daphnia magna*. The daphnids were obtained from a commercial fish farm in Manamangalam, Kancheepuram District, Tamil Nadu, India, and were held at the test facility in a glass aquarium. The holding water and the length of the holding period were not specified. Prior to test start, gravid daphnids were transferred from the aquarium to a glass jar containing the culture water and held for 24 hours. Neonates were then removed from the jar using a micropipette and transferred to the test chambers (glass jars). The neonates were not fed during the test.

The culture water was well water (no further description was provided). To prepare the water, 50 g of dry, powdered cow dung was added to one liter of well water. The prepared water was intensely aerated for 5 days and then filtered. The filtered water was then diluted (dilution rate not provided) with distilled water, and a pinch of yeast powder was added.

The test concentrations were based on the results of a range finding study. In the range finding study, daphnids were exposed to 1, 10, or 20 mg of Homobrassinolide Technical/L for 48 hours, and the number of immobilized daphnids was 0/10, 6/10, and 10/10, respectively. The nominal test solution concentrations for the main study were 0, 5, 6.5, 8.5, 11.0, and 14.3 mg of Homobrassinolide Technical/L.

Due to the low solubility of the test material in water, it was dissolved in acetone (volume not provided) and then diluted with water (unspecified) to prepare a stock solution. The appropriate volume of stock solution was then transferred to the test chambers containing aerated well water (volume/depth not provided) and the test solutions were mixed thoroughly. A solvent control group was exposed to acetone (0.1 mL/L of dilution water) only. The test consisted of 6 groups of 5 daphnids each, with each group replicated four times (20 daphnids/group).

The laboratory was controlled for temperature and humidity, and the photoperiod was 12 hours light/12 hours darkness. After 48 hours, the number of immobilized daphnids in each test chamber was counted, and probit analysis was used to calculate the EC_{50}.

Results Summary

The initial and final water temperatures were 20°C and 20.4°C, respectively, and the dissolved oxygen concentrations were 8.2 mg/L and 8.4 mg/L (percent saturation was not provided). The

initial and final solution pHs were 7.4 and 7.5, respectively, and total water hardness as $CaCO_3$ was 265 mg/L at test start and 271 mg/L at test end.

Immobility results are summarized in Table 1. The nominal 48-hr EC_{50} value was 8.90 mg/L, with a 95% confidence interval of 8.47 to 9.34 mg/L.

Table 1. Immobilization of D. magna exposed to Homobrassinolide Technical for 48 hours

Nominal Homobrassinolide Technical Concentration (mg/L)	No. Daphnids exposed	No. daphnids immobilized at test end	Mean percent immobilized
0	20	0/20	0
5	20	0/20	0
6.5	20	4/20	20
8.5	20	8/20	40
11.0	20	14/20	70
14.3	20	20/20	100

Data from p. 18, MRID 47185130

Study Author's Conclusions

The study author concluded that the nominal 48-hr EC_{50} value was 8.90 mg/L, with a 95% confidence interval of 8.47 to 9.34 mg/L.

Reviewer's Conclusion

It is unclear to the reviewer if the test water was identical to the culture water, and the dilution water used in the test was not described. The percent oxygen saturation in the test chambers was not provided. The photoperiod was not 16 hrs/8 hrs as specified in the OPPTS guideline. Although the nominal concentration of acetone in the solvent control test solutions was reported, the concentration of acetone in the test material solutions was not. Furthermore, the concentration of the test material in the test solutions was not verified. This study is unacceptable.

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Fish Acute Toxicity Test, Freshwater and Marine (OPPTS 850.1075)

MRID 47185129

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: Anthony Q. Armstrong
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Fish Acute Toxicity Test, Freshwater and Marine (OPPTS 850.1075)
MRID NO:	47185129
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	9509
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	International Institute of Biotechnology and Toxicology (IIBAT), Padappai-601 301, Kancheepuran District, Tamil Nadu, India
TITLE OF REPORT:	Homobrassinolide Technical. Biochemical Pesticides Nontarget Organisms Toxicology Data. Acute Immobilisation Test to Freshwater Fish, *Poecilia reticulata* and *Brachydanio rerio*.
AUTHORS:	Chittabubu, R.A.
STUDY COMPLETED:	May 9, 2006
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study was conducted in accordance with 40 CFR Part 1060.
STUDY SUMMARY:	In a 96-hour static renewal acute toxicity test, groups of guppies (*Poecilia reticulata*) were exposed to test solutions containing nominal concentrations of 0 (solvent control), 5, 8.8, 15.4, 26.8, or 46.9 mg/L Homobrassinolide Technical (a.i., 80.2% w/w homobrassinolide), and groups of zebra fish (*Brachydanio rerio*) were exposed to nominal concentrations of 0 (solvent control), 5, 7.5, 11.3, 16.9, or 25.3 mg/L. *P. reticulata* in the 26.8 and 46.9 mg/L groups exhibited loss of equilibrium and rapid opercular movement after 72 hours. At test end, cumulative mortality

	in the *P. reticulata* groups was 0, 0, 10, 20, 60, and 80%, respectively, and the nominal 96-hour LC_{50} was 24.56 mg/L, with 95% confidence limits of 20.44 - 28.68 mg/L. *B. rerio* in the 16.9 mg/L group exhibited loss of equilibrium after 72 hours, and *B. rerio* in the 25.3 mg/L group exhibited lateral lying on the bottom of the test chamber and rapid opercular movement after 72 hours. Cumulative mortality in the *B. rerio* groups was 0, 0, 10, 30, 60, and 90%, respectively, and the nominal 96-hour LC_{50} was 14.38 mg/L, with 95% confidence limits of 12.79– 15.98 mg/L.
CLASSIFICATION:	**Unacceptable**. The actual concentration of Homobrassinolide Technical in the test solutions was not reported.

Test Material

Homobrassinolide Technical, reported purity 80.2%, Lot No. not provided, a cream colored powder obtained from the study sponsor with an expiration date of September 23, 2006. A certificate of analysis is provided on p. 37 of MRID 47185129. After receipt at the testing facility, the test material was stored in the utility room.

Test Methods

A 96-hour static renewal acute toxicity test was conducted to determine the acute toxicity of the test material to the guppy (*Poecilia reticulata*) and the zebra fish (*Brachydanio rerio*). The fish were obtained from a commercial fish farm, and held for 12 days in aquaria containing drinking well water. The fish were 2 ± 1 cm in length, and the loading rate was ≤ 1 g of fish/L of water. After the holding period, the fish were acclimated to laboratory conditions for 7 days, during which they were fed daily with a commercial fish food. Feeding was stopped 24 hours prior to test start. No mortality occurred during the acclimation period.

Prior to the study, a range finding study was conducted exposing groups of each species of fish to nominal concentrations of 1, 50, or 100 mg/L of the test material for 48 hours. After 24 hours, mortality of both species in the 100 mg/L group was 100%. After 48 hours, mortality in the 50 mg/L group was 30% in *P. reticulata* and 60% in *B. rerio*. All fish in the 1 mg/L group survived.

In the main study, groups of 10 *P. reticulata* were exposed to nominal concentrations of 0 (solvent control), 5, 8.8, 15.4, 26.8, or 46.9 mg/L Homobrassinolide Technical for 96 hours. Groups of 10 *B. rerio* were exposed to 0 (solvent control), 5, 7.5, 11.3, 16.9, or 25.3 mg/L for 96 hours. Due to the low solubility of the test material in water, it was dissolved in acetone (volume not provided) and then diluted with water (unspecified) to prepare a stock solution. Known volumes (not specified) of the stock solution were then each mixed with 20 L of drinking well water to achieve the desired test material concentrations. The amount of acetone used in the solvent control group was not specified. The fish were exposed to the test solutions in 20-L glass aquaria (volume/depth of the test solutions in the aquaria was not reported). The test solutions were renewed daily. MRID 47185129 states that a sample from the exposure media was checked for stability and homogeneity

and found to be within permissible limits (no further information provided). Environmental conditions of the testing room were not reported.

The fish were observed for mortality and abnormal behavior after 3 and 6 hours of exposure on day 1, and every 24 hours thereafter. Fish were considered dead if they did not react when touched on the caudal peduncle. At test end, the LC_{50} was calculated using probit analysis.

Results Summary

During the test, the solution temperature ranged from 20 to 21.5°C, and pH ranged from 7.1 to 7.3. Dissolved oxygen ranged from 7.7 to 7.9 mg/L (percent saturation was not provided), and total hardness as $CaCO_3$ ranged from 265 to 282 mg/L.

P. reticulata exhibited loss of equilibrium and rapid opercular movement at 72 hours in the 26.8 and 46.9 mg/L groups. *P. reticulata* mortality is summarized in Table 1. Since the cumulative maximum mortality at 24 and 48 hours was 20% and 40% respectively, the 24- and 48- hour LC_{50}s could not be calculated using probit analysis, and were considered to be >46.9 mg/L. The 72-hour LC_{50} was 35.70 mg/L, with 95% confidence limits of 25.72 – 45.70 mg/L. The 96-hour LC_{50} was 24.56 mg/L, with 95% confidence limits of 20.44 – 28.68 mg/L.

Table 1. Mortality of *P. reticulata* exposed to Homobrassinolide Technical for 96 hours

Nominal Homobrassinolide Technical Concentration (mg/L)	No. fish tested	24 hrs		48 hrs		72 hrs		96 hrs	
		M	CM	M	CM	M	CM	M	CM
0	10	0	0	0	0	0	0	0	0
5	10	0	0	0	0	0	0	0	0
8.8	10	0	0	0	0	0	0	1	10
15.4	10	0	0	1	10	1	20	0	20
26.8	10	1	10	2	30	1	40	2	60
46.9	10	2	20	2	40	2	60	2	80

Data from p. 23, MRID 47185129
M = mortality
CM = cumulative mortality (%)

B. rerio exhibited loss of equilibrium at 72 hours in the 16.9 mg/L group, and lateral lying on the bottom of the test chamber and rapid opercular movement at 72 hours in the 25.3 mg/L group. *B. rerio* mortality is summarized in Table 2. Since the cumulative maximum mortality at 24 and 48 hours was 20% and 40% respectively, the 24- and 48 hour LC_{50}s could not be calculated using probit analysis, and were considered to be >25.3 mg/L. The 72-hour LC_{50} was 20.83 mg/L, with 95% confidence limits of 16.43 – 25.23 mg/L. The 96-hour LC_{50} was 14.38 mg/L, with 95% confidence limits of 12.79– 15.98 mg/L.

Table 2. Mortality of *B. rerio* exposed to Homobrassinolide Technical for 96 hours									
Nominal Homobrassinolide Technical Concentration (mg/L)	No. fish tested	24 hrs		48 hrs		72 hrs		96 hrs	
		M	CM	M	CM	M	CM	M	CM
0	10	0	0	0	0	0	0	0	0
5	10	0	0	0	0	0	0	0	0
7.5	10	0	0	0	0	1	10	0	10
11.3	10	0	0	1	10	1	20	1	30
16.9	10	1	10	2	30	1	40	2	60
25.3	10	2	20	2	40	2	60	3	90

Data from p. 24, MRID 47185129
M = mortality
CM = cumulative mortality (%)

Study Author's Conclusions

The study author concluded that the 24-, 48-, 72-, and 96-hour LC_{50}s for *P. reticulata* exposed to HomoBrassinolide Technical, were >46.9, >46.9, 35.70, and 24.56 mg/L, respectively. For *B. rerio* exposed to HomoBrassinolide Technical, the 24-, 48-, 72-, and 96-hour LC_{50}s were >25.3, >25.3, 20.83, and 14.38 mg/L, respectively.

Reviewer's Conclusion

The concentration of acetone in the solvent control and test material solutions was not reported. The percent oxygen saturation in the test chambers was not provided. The photoperiod during the test was not reported. Although it was stated that a "sample from the exposure media was checked for stability and homogeneity," no analytical results were provided. This study is unacceptable.

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for Nontarget Plant Studies (OPPTS 850.4000)

MRID 47185145

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: Anthony Q. Armstrong
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for Nontarget Plant Studies (OPPTS 850.4000)
MRID NO:	47185145
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-ECOTOX-55
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. Nontarget Plant Studies.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	The information submitted is sufficient to support the requested waiver for nontarget plant studies.

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for Nontarget Plant Studies (OPPTS 850.4000).

Registrant's Justification

Homobrassinolide Technical is a compound that stimulates plant growth and development. Small amounts of homobrassinolide occur naturally in all plant parts, and it is unlikely that exogenous application would cause any adverse effects to nontarget plant species. There are no published reports indicating that homobrassinolide is phytotoxic to plants. Additionally, the anticipated application rate of homobrassinolide in end use products is very low, 20 g/A. This corresponds to slightly less than 1 mg/ft^2, a negligible amount that is unlikely to have any deleterious effects on nontarget plants.

Reviewer's Conclusion

The information submitted is sufficient to support the requested waiver for nontarget plant studies. The registrant calculated the anticipated application rate of homobrassinolide as follows: 20 g/A = 20,000 mg/43,560 ft^2 = 0.918 mg/ft^2. The reviewer calculates the application rate to be even lower, 0.459 mg/ft^2 .

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for Avian Acute Oral Toxicity (OPPTS 850.2100)

MRID 47185143

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: Anthony Q. Armstrong
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: Lee Ann Wilson
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

Waiver Request

The registrant is requesting a waiver of the data requirement for Avian Acute Oral Testing (OPPTS 850.2100).

Registrant's Justification

In an acute oral toxicity study in rats, the LD_{50} for Homobrassinolide Technical was >5000 mg/kg (Toxicity Category IV). In an acute oral toxicity study in mice, the LD_{50} for Homobrassinolide Technical was >5000 mg/kg (Toxicity Category IV). In a 90-day oral toxicity study in rats, the NOAEL for Homobrassinolide Technical was 1000 mg/kg/day. Based on these studies, Homobrassinolide Technical is expected to have very low toxicity to avian species. Also, since homobrassinolide occurs naturally in plants, avian species are already exposed to the substance.

The anticipated application rate of homobrassinolide in end use products is very low, 20 g/A. This corresponds to slightly less than 1 mg/ft^2, a negligible amount that is unlikely to have any deleterious effects on avian species. Additionally, homobrassinolide will be applied as a liquid spray, not in a granular form, making potential exposure of avian species unlikely.

Reviewer's Conclusion

The information submitted is sufficient to support the requested waiver for Avian Acute Oral Toxicity testing. The registrant calculated the anticipated application rate of homobrassinolide as follows: 20 g/A = 20,000 mg/43,560 ft^2 = 0.918 mg/ft^2. The reviewer calculates the application rate to be even lower, 0.459 mg/ft^2 .

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for Avian Acute Dietary Toxicity (OPPTS 850.2200)

MRID 47185144

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: Anthony Q. Armstrong
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for Avian Acute Dietary Toxicity (OPPTS 850.2200)
MRID NO:	47185144
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-ECOTOX-54
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. Avian Acute Dietary.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	The information submitted is sufficient to support the requested waiver for Avian Acute Dietary Toxicity testing.

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for Avian Acute Dietary Testing (OPPTS 850.2200).

Registrant's Justification

In an acute oral toxicity study in rats, the LD_{50} for Homobrassinolide Technical was >5000 mg/kg (Toxicity Category IV). In an acute oral toxicity study in mice, the LD_{50} for Homobrassinolide Technical was >5000 mg/kg (Toxicity Category IV). In a 90-day oral toxicity study in rats, the NOAEL for Homobrassinolide Technical was 1000 mg/kg/day. Based on these studies, Homobrassinolide Technical is expected to have very low toxicity to avian species. Also, since homobrassinolide occurs naturally in plants, avian species are already exposed to the substance.

The anticipated application rate of homobrassinolide in end use products is very low, 20 g/A. This corresponds to slightly less than 1 mg/ft^2, a negligible amount that is unlikely to have any deleterious effects on avian species. Additionally, homobrassinolide will be applied as a liquid spray, not in a granular form, making potential exposure of avian species unlikely.

Reviewer's Conclusion

The information submitted is sufficient to support the requested waiver for Avian Acute Dietary Toxicity testing. The registrant calculated the anticipated application rate of homobrassinolide as follows: 20 g/A = 20,000 mg/43,560 ft^2 = 0.918 mg/ft^2. The reviewer calculates the application rate to be even lower, 0.459 mg/ft^2 .

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for *in vitro* Mammalian Cell Gene Mutation Testing
(OPPTS 870.5300)

MRID 47185132

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T.

Signature: Sylvia Milanez
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for *in vitro* Mammalian Cell Gene Mutation Testing (OPPTS 870.5300)
MRID NO:	47185132
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-TOX-42
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. *In Vitro* Mammalian Cell Gene Mutation Test.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	The information submitted is sufficient to support the requested waiver for *in vitro* mammalian cell gene mutation testing.

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for *in vitro* Mammalian Cell Gene Mutation Testing (OPPTS 870.5300).

Registrant's Justification

In a reverse mutation study (MRID 47208904), Homobrassinolide Technical at dose levels of 0.03 to 0.05 μg/plate was non-mutagenic in *Salmonella typhimurium* strains TA1537, TA1535, TA98, and TA100.

In a chromosomal aberration test (MRID 47208905), Homobrassinolide Technical did not have the potential to induce chromosome aberrations in mice treated up to a single oral dose of 2000 mg/kg body weight.

In a micronucleus test (MRID 47185127), Homobrassinolide Technical did not have micronucleus induction potential in mice after two days of oral dosing up to a level of 2000 mg/kg body weight.

Based on the results of these three studies, the registrant concluded that Homobrassinolide Technical has no potential for genotoxicity.

Reviewer's Conclusion

The ORNL reviewer for the reverse mutation study cited above found it to be unacceptable. However, the information submitted is sufficient to support the requested waiver for *in vitro* mammalian cell gene mutation testing.

4. **Test organisms:** ***S. typhimurium* strains *(TA#) and E. coli strains***

[ ] TA97	[X] TA98	[X] TA100	[ ] TA102	[ ] TA104
[X] TA1535	[X] TA1537	[ ] TA1538	[ ] *E. coli* WP2 *uvrA*	[ ]
[ ]	[ ]	[ ]	[ ]	[ ]
[ ]	[ ]	[ ]	[ ]	[ ]

Properly maintained? [X] Yes [ ] No

Checked for appropriate genetic markers (*rfa* mutation, R factor)? [X] Yes [ ] No

5. **Test compound concentrations used:** (Triplicate plating):

Nonactivated conditions: 0, 0.03, 0.06, 0.12, 0.25 and 0.5 µg/plate in all strains
Activated conditions: 0, 0.03, 0.06, 0.12, 0.25 and 0.5 µg/plate in all strains

B. TEST PERFORMANCE:

1. **Type of *Salmonella* assay:**

[X] Standard plate test
[ ] Pre-incubation 20 minutes
[ ] "Prival" modification *(i.e. azo-reduction method)*
[ ] Spot test
[ ] Other

2. **Protocol:**

For each test dose, for the positive controls, and the vehicle controls, 2 mL of top agar (previously melted and cooled to 45°C ± 2°C) was added to each of two sterile test tubes. Then 500 µL of 5% S9 mix was added to one of tubes and 500 µL of 0.2M phosphate buffer was added to the other tube. To each pair of tubes was added 100 µL of the appropriately diluted test article or positive control chemical dissolved in DMSO, or DMSO alone for the vehicle control. Finally 100 µL of standard bacterial suspension was added to both tubes and mixed thoroughly. This top agar was added to Petri plates containing 25 mL of Minimal Glucose Agar. The plates were incubated at 37°C = 1°C for 72 hours and then the number of mutant colonies was counted. Three replicates were used for each treatment condition.

3. **Statistical analysis:**

Simple linear regression analysis was performed on all tester strains, without and with activation, to determine if there was any dose dependent-increase in the number of revertant colonies.

4. **Evaluation criteria:**

A positive result was defined as a statistically significant, dose-dependent increase in the number of histidine independent revertants, with at least one dose level inducing a revertant frequency that was at least two-fold higher than the spontaneous solvent control value. If the test article did not induce a statistically significant, dose-dependent increase in revertant

frequency, but did induce a revertant frequency at one dose level that was two-fold or more greater than the spontaneous control value, the result was considered equivocal. A negative result was defined as the absence of a statistically significant or dose-dependent increase in the number of histidine independent revertants.

II. RESULTS:

A. MUTAGENICITY ASSAY:

A summary of the *in vitro* bacterial gene mutation assay for brassinosteroids technical in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, without or with activation, is presented in Table 1. The average number of mutants found on three plates per treatment group is shown. No increase in the number of revertants per plate was seen at any test article concentration without or with S9-mix. The solvent and positive control values were appropriate. Regression analysis of the mutants in each treatment group, without or with activation, showed no significant dose-dependency.

TABLE 1. Summary of mutations induced in four tester strains of *Salmonella typhimurium* without and with activation following exposure to brassinosteroids technical.

Treatment (Dose in µg/plate)	Number of revertant colonies per plate (mean ± standard deviation)			
	TA98	TA100	TA1535	TA1537
Without S9-mix				
Solvent (DMSO)	37.7 ± 1.5	137.7 ± 1.5	14.0 ± 1.0	14.0 ± 1.0
Brassinosteroids Technical:				
0.03	37.3 ± 2.1	141.0 ± 1.0	15.0 ± 1.0	15.3 ± 1.5
0.06	38.7 ± 1.2	144.7 ± 0.6	12.7 ± 1.2	14.3 ± 2.5
0.12	35.0 ± 2.0	136.7 ± 1.5	16.0 ± 1.0	16.3 ± 0.6
0.25	36.3 ± 0.6	137.3 ± 0.6	13.0 ± 1.0	10.3 ± 2.5
0.5	32.3 ± 0.6	141.7 ± 1.5	14.3 ± 0.6	11.6 ± 1.5
Positive control (µg/plate):	4NPD (20)	SA (10)	SA (10)	9AA (150)
Average mutants/plate:	3290.3 ± 126.6	690.3 ± 169.9	1309.7 ± 111.0	1129.3 ± 104.0
With S9-mix				
Solvent (DMSO)	43.7 ± 0.6	167.3 ± 2.1	18.0 ± 1.0	16.7 ± 1.5
Brassinosteroids Technical:				
0.03	47.0 ± 1.0	171.7 ± 1.5	19.7 ± 1.5	18.0 ± 1.0
0.06	43.0 ± 1.0	165.3 ± 2.5	14.7 ± 1.5	19.7 ± 1.5
0.12	45.0 ± 2.0	170.3 ± 1.5	16.7 ± 1.5	14.7 ± 1.5
0.25	47.3 ± 1.2	165.3 ± 2.5	12.7 ± 3.8	17.3 ± 1.5
0.5	41.3 ± 1.5	165.3 ± 2.5	14.7 ± 1.5	11.3 ± 1.2
Positive control (µg/plate):	2AF (20)	2AF (20)	2AF (20)	2AF (20)
Average mutants/plate:	3099.7 ± 159.3	569.3 ± 31.3	74.3 ± 10.0	76.7 ± 4.9

Data summarized from Table 1 on pages 23–26 of MRID 47208904.
All plating was in triplicate.
4NPD = 4-Nitro-1, 2-phenylene diamine
SA = Sodium azide
9AA = 9-Aminoacridine
2AF = 2-Aminofluorene

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

The investigator concluded that brassinosteroids technical at all tested dose levels (0.03, 0.06, 0.12, 0.25, and 0.5 µg/plate) was non-mutagenic in all four of the *S. typhimurium* strains tested (TA98, TA100, TA1535, and TA1537), both in the absence and in the presence of 5% S9 mix.

B. REVIEWER COMMENTS:

The reviewer agrees with the investigator's conclusions as far as they go. However, the doses that were tested were extremely low. The highest dose tested was 10^4 times lower than the limit dose of 5000 µg/plate recommended by the Guidelines. There was no mention of any toxicity caused by the test article, without or with activation, so it is unacceptable to have used such a low dose range (0.03 to 0.5 µg/plate). In addition, only 4 of the recommended 5 strains of bacteria were tested, and almost no information was provided on the S9 fraction used for activation. Other than indicating that liver was the source of the S9, no other information was provided on the animal species or whether induction was used. Also, the description of the S9 mix was incomplete. While the investigator did measure the optical densities of the bacterial cultures (Appendix 4, page 31 of MRID 47208904) in what was referred to as a "cell viability test," these optical densities were only crudely related to actual bacterial counts. Furthermore, it was not clear that the bacterial cultures used in the "cell viability test" were the same as those used for the mutagenicity assay. No quantitative measurement could be made of the number of revertants/ 10^6 viable bacterial cells. No historical control data was presented. This is an **Unacceptable** study.

C. STUDY DEFICIENCIES:

Major deficiencies are discussed above in the reviewer's comments.

DATA EVALUATION RECORD

BRASSINOSTEROIDS TECHNICAL

STUDY TYPE: MAMMALIAN BONE MARROW CHROMOSOMAL ABERRATION TEST; OPPTS 870.5385 [§84-2]
MRID 47208905

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Work Assignment #07-080

Primary Reviewer:
Gary Sega, Ph.D.

Signature: Gary Sega
Date: FEB 21 2008

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T.

Signature: Sylvia Milanez
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Kimberly G. Slusher, M.S.

Signature: Kimberly G. Slusher
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

Secondary Reviewer: **Date:**_____

DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte chromosomal aberration assay in mice OPPTS 870.5385 [§84-2]; OECD 475.

EPA Reg. No.: 69361-RT **Product Name**: Brassinosteroids Technical
DECISION: 381556 **DP BARCODE:** 347313

TEST MATERIAL (PURITY): Brassinosteroids Technical (85.1%)

SYNONYMS: None provided.

CITATION: Prabakaran, P. (1998) Homobrassinolide Technical. Biochemical Pesticides Toxicology Data. Chromosomal aberration in mice. Jai Research Foundation, Valvada 396 108, Dist. Valsad, Gujarat, India. JRF Report No. 272, September, 1998. MRID 47208905. Unpublished.

SPONSOR: Godrej Agrovet Ltd., Pirojshah Nagar, Eastern Express Highway, Vikhroli, Mumbai – 400 079 INDIA.

EXECUTIVE SUMMARY:

In a bone marrow chromosome aberration assay, four Swiss albino male mice per dose were exposed to Brassinosteroid Technical (purity 85.1%), batch no. 970004, by gavage, at doses of 0, 500, 1000, or 2000 mg/kg bw, using carboxy methyl cellulose as the vehicle. All animals were sacrificed 24 hours after treatment. Four hours prior to sacrifice the animals were treated with colchicine to arrest cells in metaphase. No significant changes in body weights of the treated animals were observed during the period of treatment at any dose level nor were there any clinical signs of toxicity observed at any dose level. Mitomycin C, the positive control, was dissolved in distilled water and administered as a single dose via i.p. injection at 4 mg/kg bw in a volume of 10 mL/kg bw.

Brassinosteroid Technical was tested up to a limit dose of 2000 mg/kg bw. In the cytogenetic assay, there were no biologically or statistically significant increases in the aberration frequencies over the vehicle control value at any test article dose and no effect on the mitotic index was seen. The vehicle and positive control induced appropriate responses. **There was no evidence of chromosomal aberrations induced over background.** A number of deficiencies were identified that did not invalidate the study results: only male mice were tested without data showing that there was no substantial difference in toxicity between the sexes, no justification was given for the use of carboxymethyl cellulose as the vehicle, the stability of the test article was not reported, only four animals were used per group instead of five, and no historical control

data were presented. Only one sampling time was used, but since the limit dose was tested, and there was no hint of an increase in the chromosome aberration frequency at any test dose, it is considered unlikely that a significant increase in the chromosome aberration frequency would be seen at a second sampling time.

This study is classified as **Acceptable** and in general satisfies the guideline requirement for Test Guideline OPPTS 870.5385; OECD 475 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided. A signed and dated Management Statement, equivalent to a Quality Assurance statement was included.

I. MATERIAL AND METHODS

A. MATERIALS:

1. **Test Material:** Brassinosteroid Technical

Description:	Light yellowish-brown colored powder.
Lot/Batch No.:	970004
Purity:	85.1%
Chemical Name:	Not reported.
CAS No. of TGAI:	Not reported.

2. **Control Materials:**

Negative control (if not vehicle):		**Final Volume:**	**Route:**
Vehicle:	Carboxy methyl cellulose	**Final Volume:** 10 mL/kg bw	**Route:** oral by metal cannula
Positive control:	Mitomycin-C (MMC), dissolved in distilled water.	**Final Dose(s):** 4 mg/kg bw	**Route:** i.p.

3. **Test animals:**

Species:	Mouse
Strain:	Swiss albino
Age/weight at study initiation:	Approximately 7 weeks of age / males only: 32 to 36 g
Source:	Jai Research Foundation
No. animals used per dose	4 males; 0 females
Properly Maintained?	Yes

4. **Test compound administration:**

	Dose Levels	Final Volume	Route
Toxicity tests:			
None reported			
Main Study:	0, 500, 1000, 2000 mg/kg bw	10 mL/kg bw	Oral, via metal cannula

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

a. Test compound:

Dosing:	x	once		twice (24 hrs apart)			Other (high dose only)			
Sampling (after last dose):	x	24 hr		12 hr		24 hr		48 hr		72 hr
Other:										

b. Negative and/or vehicle control:

Dosing:	x	once		twice (24 hrs apart)			Other			
Sampling (after last dose):	x	24 hr		12 hr		24 hr		48 hr		72 hr
Other:										

c. Positive control:

Dosing:	x	once		twice (24 hrs apart)			Other			
Sampling (after last dose):	x	24 hr		12 hr		24 hr		48 hr		72 hr
Other:										

2. Preparation of the Bone Marrow:

On the day following treatment with the test article, four hours prior to sacrifice, all animals received an i.p. injection of colchicine (4 mg/kg bw). Mice were sacrificed by cervical dislocation, femurs from the animals were recovered and the epicondyle tips removed. The bone marrow cells were flushed directly into centrifuge tubes with a 1 mL syringe and a 24 G needle using 5 mL of phosphate buffered saline (pH 7.4). The bone marrow suspension was mixed vigorously to disassociate the cells and centrifuged for 10 minutes at 2000 rpm. The supernatant was discarded and freshly prepared hypotonic 0.075 M potassium chloride was added to the residual cell pellet and mixed thoroughly. The cells were incubated at 37°C for 30 minutes, then recentrifuged at 2000 rpm for 10 minutes and the supernatant was discarded. Fresh, chilled Carnoy's fixative (methanol:glacial acetic acid 3 : 1) was added (5 mL) to the cell pellet. The tubes were refrigerated at 4°C for a minimum of 12 hours, then centrifuged. The supernatant was discarded and cell pellet was resuspended in fresh Carnoy's fixative. The tubes were again centrifuged and the supernatant was discarded leaving 0.5 mL of fixative with the cell pellet. Two slides for each animal were prepared by dropping 0.5 mL of fixed cell suspension drop by drop on pre-cleaned, ice-chilled slides (stored in methanol:distilled water 1 : 1). The slides were dried on a slide warmer, labelled with test number, animal number and slide number, and stained with 5% Giemsa in phosphate buffer for 10 minutes. One of the two slides prepared from each animal was used for scoring and the other was kept as a reserve.

3. ANALYSIS OF METAPHASE CELLS:

Evaluation of the slides was performed using a Nikon Optiphot-2 optical microscope. A minimum of 500 cells/animal were counted to determine the mitotic index (MI), using the formula: MI = [Number of metaphases/ Number of cells counted] x 100.

A minimum of 100 well-spread metaphases per animal were scored using 100x oil immersion objectives. Gaps, breaks, fragments, and numerical (ploidy) anomalies and pulverizations were recorded. The number of cells with one or more chromosome aberrations, excluding gaps, were recorded to calculate the percent of aberrant cells.

4. EVALUATION / ACCEPTANCE CRITERIA:

No description was given of the types of aberrations scored, however, the results include the finding of chromatid breaks, chromatid breaks, chromosomal breaks, fragments, pulverization and numerical aberrations. No acceptance criteria were presented.

5. Evaluation of Results / Statistical Methods:

The data on body weight, mitotic index and % aberrant cells were analyzed statistically, using Student's t-test.

II. RESULTS:

No significant changes in body weights of the treated animals were observed at any dose level. There were no clinical signs of toxicity observed at any dose level or at any time during the study. There was no reduction in the mitotic index of the cells from the treated mice at any dose level compared to the vehicle control (Table 1). The percent of aberrant cells from mice treated with the test article up to a dose of 2000 mg/kg b.w. did not differ significantly from the vehicle control (Table 1). The structural aberrations observed were mainly chromatid breaks and numerical anomalies (reported for the individual animals in Appendix 3, page 21 of MRID 47208905). The positive control mutagen (Mitomycin C) produced chromatid breaks, chromosomal breaks, fragments, pulverization and numerical aberrations and overall showed a statistically significant increase in the aberration frequency (10.75% vs 0.75% for the vehicle controls). The vehicle control value was appropriate. No historical control values were presented.

TABLE 1. Summary of chromosomal aberrations found in bone marrow of male mice after treatment with brassinosteroids technical.

Group	Dose (mg/kg b.w.)	Mean Mitotic Index (%) ± S.D. [a]	Mean % Aberrant Cells ± S.D.
Vehicle control (carboxymethyl cellulose)	0	1.674 ± 0.376	0.750 ± 0.957
Brassinosteroids Technical	500	1.729 ± 0.307	0.750 ± 0.957
	1000	1.664 ± 0.427	0.750 ± 0.957
	2000	2.069 ± 0.203	0.750 ± 0.500
Positive control (MMC)	4	2.067 ± 0.290	10.750 ± 0.957 *

Data obtained from MRID 47208905, Table 2 on page 18.
[a] S.D. = standard deviation.
*Statistically significantly higher than vehicle control, $p < 0.05$ by Student's t test.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

The investigator concluded that under the test conditions used, brassinosteroids technical does not induce chromosomal aberrations in bone marrow cells of mice treated with a single oral dose of the test article up to 2000 mg/kg b.w.

B. REVIEWER COMMENTS:

Presentation of the methods used and discussion of the results obtained were minimal in this report. However, based on the data presented, the reviewer agrees with the investigator's conclusions. The test article was studied up to the limit dose required by the Guidelines. All three doses of the test article produced an average aberration frequency that was equal to the negative vehicle control. The positive and negative controls gave appropriate responses.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for OPPTS 870.5385; OECD 474 for *in vivo* cytogenetic mutagenicity data.

C. STUDY DEFICIENCIES:

A number of deficiencies were identified that did not invalidate the study results. Female mice were tested, and no data or references were presented to show that there was no substantial difference in toxicity of the test article between the sexes. No justification was given for the use of carboxymethyl cellulose as the vehicle. The stability of the neat test article and its stability in the vehicle were not reported. Only four animals were used/group instead of the recommended five. No historical control data were presented. Only one sampling time was used, however, since testing up to the limit dose produced no hint of an increase in chromosome aberrations, it is considered unlikely that a significant increase in the chromosome aberration frequency would be seen at a second sampling time.

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPES: Product Identity and Composition (OPPTS 880.1100)
Description of Starting Materials, Production and Formulation Process (OPPTS 880.1200)
Discussion of Formation of Impurities (OPPTS 880.1400)
Preliminary Analysis (OPPTS 830.1700)
Certified Limits (OPPTS 830.1750)
Enforcement Analytical Method (OPPTS 830.1800)
Physical and Chemical Characteristics (OPPTS 830.6302-830.7950)

MRIDs 47185101-04, 47185106-08, 47185110-17, 47208901-02

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T.

Signature: Sylvia Milanez
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.N. Wilson
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Product Identity and Composition (OPPTS 880.1100) Description of Starting Materials, Production and Formulation Process (OPPTS 880.1200) Discussion of Formation of Impurities (OPPTS 880.1400) Preliminary Analysis (OPPTS 830.1700) Certified Limits (OPPTS 830.1750) Enforcement Analytical Method (OPPTS 830.1800) Physical and Chemical Characteristics (OPPTS 830.6302-830.7950)
MRID NOS:	47185101-04, 47185106-08, 47185110-17, 47208901-02
DECISION NO:	381556
DP BARCODE:	DP347313
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
PROJECT STUDY NO:	MRID 47185101: REPAR-HBR-TECH-PC-1 MRID 47185102: HBR-03/05 MRID 47185103: REPAR-HBR-TECH-PC-2 MRID 47185104: REPAR-HBR-PC-6 MRID 47185106: REPAR-HBR-PC-8 MRID 47185107: REPAR-HBR-PC-9 MRID 47185108: REPAR-HBR-PC-10 MRID 47185110: REPAR-HBR-PC-12 MRID 47185111: REPAR-HBR-PC-13 MRID 47185112: REPAR-HBR-PC-14 MRID 47185113: REPAR-HBR-PC-15 MRID 47185114: REPAR-HBR-PC-16 MRID 47185115: REPAR-HBR-PC-17 MRID 47185116: REPAR-HBR-PC-18 MRID 47185117: REPAR-HBR-PC-3 MRID 47208901: REPAR-HBR-PC-7 MRID 47208902: REPAR-HBR-PC-11
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	MRID 47185101: N/A MRID 47185102: Reliable Analytical Laboratories, Manpada, Thane, India MRID 47185103: N/A MRIDs 47185104, 47185106-08, 47185110-16, 47208901-02: Fredrick Institute of Plant Protection and Toxicology, Tamil Nadu, India MRID 47185117: N/A
TITLE OF REPORT:	MRID 47185101: Homobrassinolide Technical. Product Identity and Composition. MRID 47185102: Homobrassinolide Technical. Product Analysis. MRID 47185103: Homobrassinolide Technical. Analysis and Certified Limits. MRID 47185104: Homobrassinolide Technical. Physical and Chemical Properties. Color. MRID 47185106: Homobrassinolide Technical. Physical and Chemical Properties. Melting Point.

MRID 47185107: Homobrassinolide Technical. Physical and Chemical Properties. Density.
MRID 47185108: Homobrassinolide Technical. Physical and Chemical Properties. Solubility in Water and Organic Solvents.
MRID 47185110: Homobrassinolide Technical. Physical and Chemical Properties. Dissociation Constant.
MRID 47185111: Homobrassinolide Technical. Physical and Chemical Properties. Octanol/Water Partition Coefficient.
MRID 47185112: Homobrassinolide Technical. Physical and Chemical Properties. pH.
MRID 47185113: Homobrassinolide Technical. Physical and Chemical Properties. Stability.
MRID 47185114: Homobrassinolide Technical. Physical and Chemical Properties. Flammability.
MRID 47185115: Homobrassinolide Technical. Physical and Chemical Properties. Corrosion Characteristics.
MRID 47185116: Homobrassinolide Technical. Physical and Chemical Properties. UV Spectral Analysis.
MRID 47185117: Homobrassinolide Technical. Physical and Chemical Characteristics.
MRID 47208901: Homobrassinolide Technical. Physical and Chemical Properties. Odor.
MRID 47208902: Homobrassinolide Technical. Physical and Chemical Properties. Vapor Pressure.

AUTHORS: MRIDs 47185101, 47185103, 47185117: Mandava, N.B.
MRID 47185102: Kaushal, R.B.
MRIDs 47185104, 47185106-08, 47185110-16, 47208901-02: Rajaraman, G.

STUDY COMPLETED: MRID 47185101: January 18, 2007
MRID 47185102: May 16, 2005
MRID 47185103: February 2, 2007
MRIDs 47185104, 47185106-08, 47185110, 47185112-14, 47185116, 47208901: July 23, 2001
MRID 47185111, 47185115, 47208902: July 5, 2001
MRID 47185117: March 21, 2007

CONFIDENTIALITY CLAIMS MRID 47185101: Confidential material is included in the confidential appendix.
MRID 47185102: Confidential material is included in the confidential appendix.
MRID 47185103: Confidential material is included in the confidential appendix.
MRID 47185104, 47185106-08, 47185110-17, 47208901-01: None.

GOOD LABORATORY PRACTICE: MRIDs 47185101, 47185103: A signed and dated GLP statement was included. The cited studies were not conducted in compliance with 40 CFR Part 160.
MRIDs 47185102, 47185115, 47185117: A signed and dated GLP statement was included. The study was not conducted in compliance with 40 CFR Part 160.
MRIDs 47185104, 47185106-08, 47185110, 47185112-14, 47185116, 47208901-02: A signed and dated GLP statement was included. The study was conducted in compliance with 40 CFR Part 160.

MRID 47185111: A signed and dated GLP statement was included. The study was conducted following OECD GLP principles, which are recognized as being equivalent to those of 40 CFR Part 160.

CONCLUSION: Homobrassinolide Technical is a technical grade active ingredient/manufacturing use product to be used only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% w/w homobrassinolide (2α,3α,22S,23S,24S)-2,3,22,23-tetrahydroxy-24-ethyl-β-homo-7-oxa-5α-cholestan-6-one). There are no intentionally-added inert ingredients in the product. Impurities in the product are [redacted] The CSF and product label are in agreement regarding the active ingredient content. The beginning materials were described, but the MSDSs submitted were not from the suppliers specified in MRID 47185101. The product is produced using an integrated process, and an adequate discussion of the formation of the impurities was provided. Acceptable results from analysis of five lots of Homobrassinolide Technical were submitted. The certified limits for the active ingredient are within the OPPTS 830.1750 guidelines. The enforcement analytical method is high performance liquid chromatography with ultraviolet detection. The physical/chemical characteristics were adequately presented, provided that the requested waivers of the one-year studies of storage stability and corrosion characteristics are granted.

CLASSIFICATION: **Acceptable** if: 1) MSDSs or specification sheets for the beginning materials are provided for the listed suppliers and 2) the waivers for the one-year studies of storage stability and corrosion characteristics are granted. A PC code will need to be assigned for homobrassinolide.

CONTAINS CONFIDENTIAL BUSINESS INFORMATION

Test Material: Homobrassinolide Technical (a.i., 80.0% homobrassinolide)

I. **PRODUCT IDENTITY AND COMPOSITION:** Homobrassinolide Technical is a technical grade active ingredient/manufacturing use product (TGAI/MUP) to be used only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% w/w homobrassinolide (2α,3α,22S,23S,24S)-2,3,22,23-tetrahydroxy-24-ethyl-β-homo-7-oxa-5α-cholestan-6-one). There are no intentionally-added inert ingredients in the product. Impurities in the product are [redacted] The CSF and product label are in agreement regarding the active ingredient content.

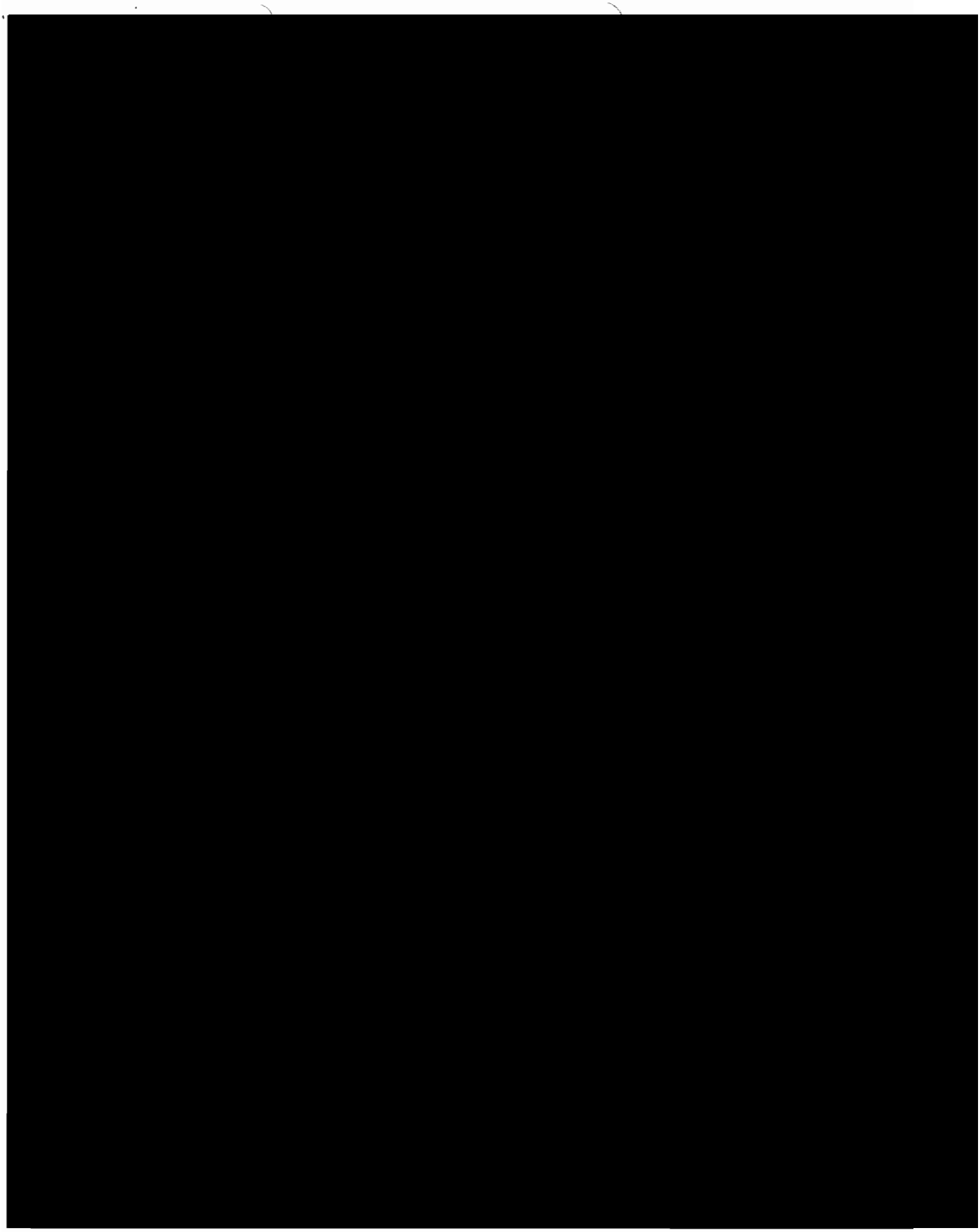
Deficiencies: A PC code will need to be assigned for homobrassinolide.

II. **DESCRIPTION OF BEGINNING MATERIALS:** The beginning materials for Homobrassinolide Technical are [redacted] MSDSs were submitted for each beginning material; however, none of the MSDSs were from the suppliers specified on pp. 21-24 of MRID 47185101.

Deficiencies: MSDSs from the suppliers of the beginning materials must be submitted.

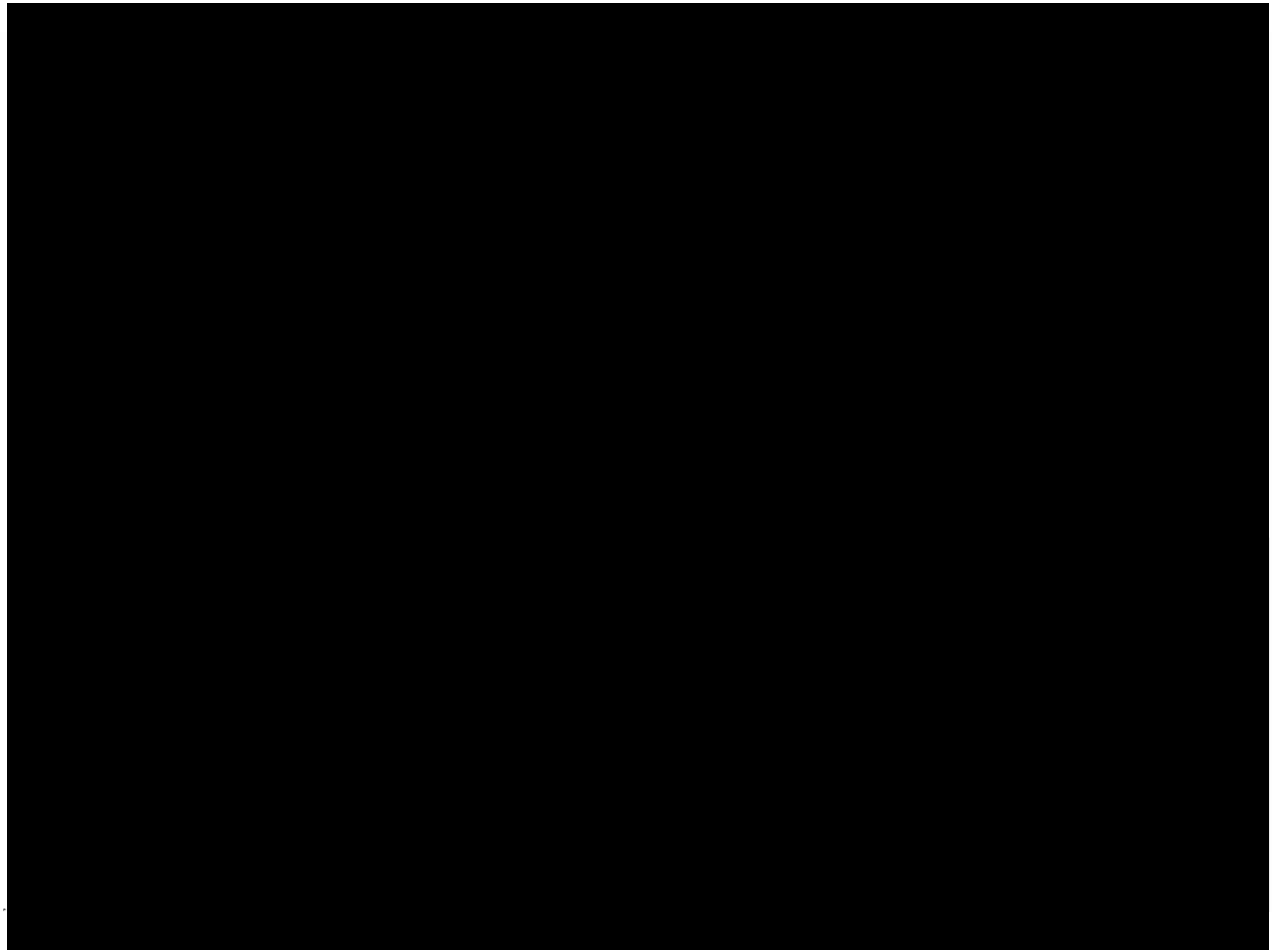
DESCRIPTION OF PRODUCTION PROCESS: Homobrassinolide Technical is produced in a batch process that [redacted]

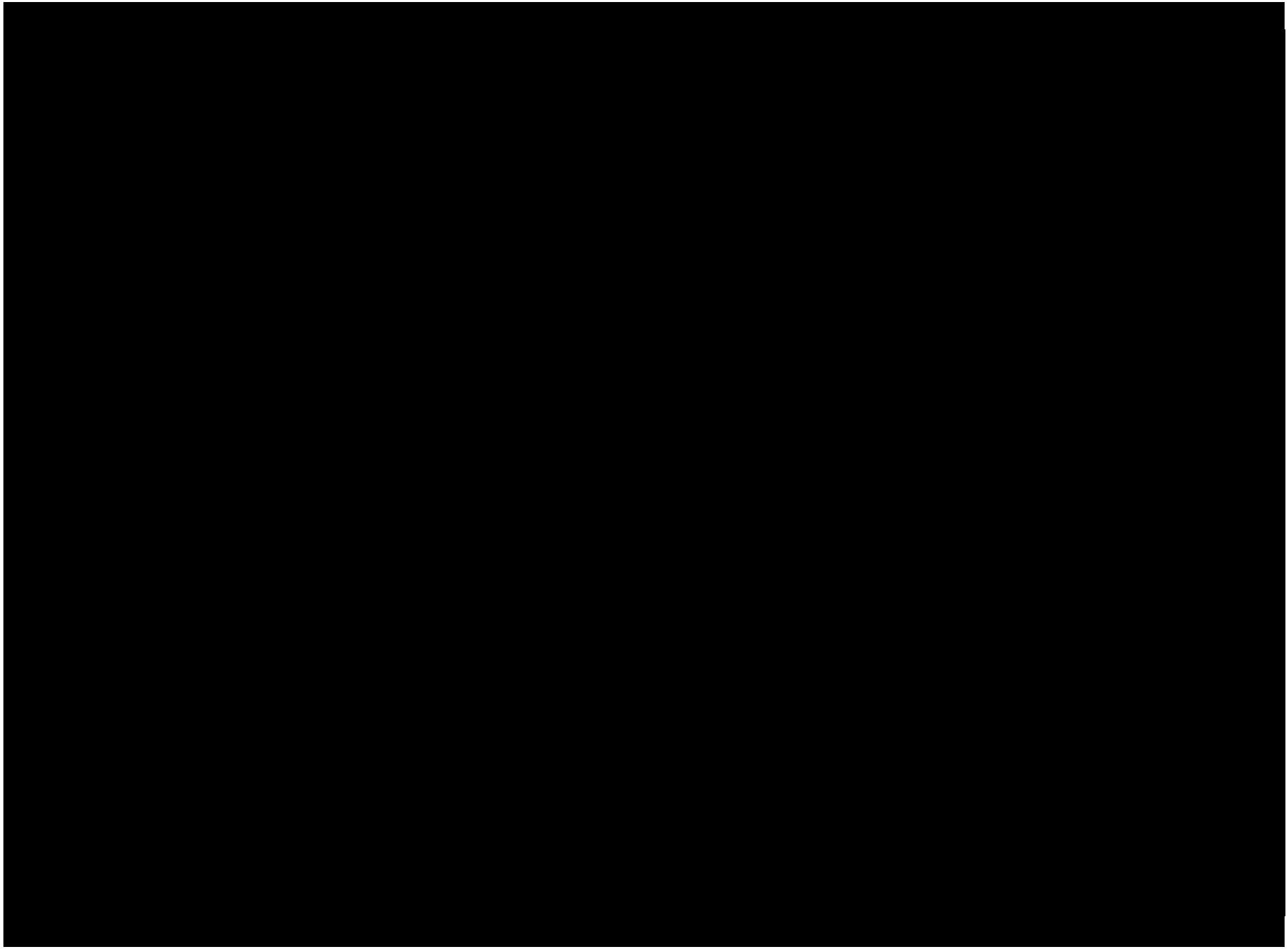
Manufacturing process information may be entitled to confidential treatment



Manufacturing process information may be entitled to confidential treatment

Manufacturing process information may be entitled to confidential treatment





[redacted]

Deficiencies: None.

III. **DISCUSSION OF FORMATION OF IMPURITIES:** Impurities potentially present in the product at a concentration >0.1% w/w are [redacted] There is no likelihood for post-production reactions between the active ingredient and the impurities. The packaging material is LDPE bags, and it is highly unlikely that any components of the packaging will enter into the product.

Deficiencies: None.

V. **PRELIMINARY ANALYSIS:** Results from a preliminary analysis of five separate batches of Homobrassinolide Technical using the enforcement analytical method were provided in MRID 47185102. The active ingredient content ranged from 79.98 to 80.87% with a mean of 80.35%, which is within the certified limits provided on the CSF. [redacted]

Deficiencies: None.

VI. **CERTIFIED LIMITS:** Table 1 lists the nominal concentrations and certified limits for the ingredients in Homobrassinolide Technical. The certified limits for the active ingredient are within the OPPTS-recommended guidelines.

TABLE 1. Nominal CSF concentrations and certified limits for Homobrassinolide Technical[a]					
Ingredients (CAS number)	PC Code	Purpose	Concentration (% by weight)		
			Nominal	Lower	Upper
Active Ingredient					
homobrassinolide (2α,3α,22S,23S,24S)-2,3,22,23-tetrahydroxy-24-ethyl-β-homo-7-oxa-5α-cholestan-6-one) (CAS No. 80483-89-2)	**Not found**	Active ingredient	80.0	76.0	84.0
[redacted]					

[a]Data from CSF

Deficiencies: None.

ENFORCEMENT ANALYTICAL METHOD: The enforcement analytical method to determine homobrassinolide in Homobrassinolide Technical is HPLC with UV detection. The instrument is a Shimadzu HPLC. The column is a Hypersil C-18 (150 mm x 4.6 mm i.d., film thickness of 5μ). Detection is at 205 nm. The mobile phase is 45:55 acetonitrile:water, and the retention time is 12-13 minutes. Sample chromatograms are provided in MRID 47185102.

Deficiencies: None.

VIII. PHYSICAL AND CHEMICAL CHARACTERISTICS:

1. **Methods**: The methods for determining the physical/chemical characteristics of Homobrassinolide Technical are given in Table 2.

2. **Results**: The physical/chemical characteristics of Homobrassinolide Technical are provided in Table 2. The registrant is requesting waivers for the one-year storage stability and corrosion characteristics tests.

Deficiencies: If the waivers for the one-year storage stability and corrosion characteristics tests are not granted, those tests will need to be conducted.

TABLE 2. Physical and Chemical Properties for Homobrassinolide Technical[a]			
Guideline Reference No./Property		**Description of Result**	**Methods**
830.6302	Color	White to pale yellow	Visual observation
830.6303	Physical State	Powder	Visual observation
830.6304	Odor	Mild, characteristic odor (1% suspension in ultrapure water)	Olfactory inspection
830.6313	Stability	Stable for 14 days at 54±2°C and 62% relative humidity. The product is packaged in HDPE containers with LDPE liners, and is not likely to contact metals during its lifetime or use.	CIPAC MT 46
830.6314	Oxidation/Reduction: Chemical Incompatibility	Not applicable, product is not intended to contact strong oxidizing or reducing agents.	
830.6315	Flammability	Not flammable at temperatures up to 100°C	OPPTS 830.6315 Closed cup
830.6316	Explodability	Product has no potential to explode.	
830.6317	Storage Stability	Stable for 14 days at 54±2°C and 62% relative humidity. **Waiver of one-year study is requested.**	CIPAC MT 46
830.6319	Miscibility	Not applicable, product is not to be mixed with petroleum solvents.	
830.6320	Corrosion Characteristics	Calculated corrosion rates based on mean weight loss of coupons exposed to the product for 14 days: Copper – 0.00143 mm/year Aluminum – 0.0540 mm/year Steel – 0.00134 mm/year. **Waiver of one-year study is requested.**	ASTM G31-72 & D1384-87
830.6321	Dielectric Breakdown Voltage	Not required for TGAI/MP	
830.7000	pH	7.65 (1 g in 100 mL of water)	CIPAC MT 75.1 Digital pH meter
830.7050	UV/Visible Absorption	λ_{max} = 1.2458 at 202 nm (10 mg in 50 mL methanol-HCl)	OECD 101
830.7100	Viscosity	Not applicable, product is a solid	
830.7200	Melting Range	116-118°C	OECD 102 Capillary tube/metal block
830.7220	Boiling Range	Not applicable, product is a solid	
830.7300	Density/Relative Density/Bulk Density	Density = 0.7 g/mL at ambient temperature	OECD 109 Pycnometer
830.7370	Dissociation Constant in Water	pKa = 6.44 at pH 6.0 6.29 at pH 6.5 6.54 at pH 7.0 7.56 at pH 7.5 7.81 at pH 8.0	OECD 112
830.7550	Partition Coefficient	Log P_{ow} = 3.96	OECD 117

830.7840	Water Solubility	3.18% in water 95.89% in acetone 99.89% in ethanol 99.97% in methanol 5.04% in hexane	CIPAC MT 157.1 & 157.2
830.7950	Vapor Pressure	Not applicable, product is a solid and does not vaporize	

[a]Data from MRIDs 47208901-02, 47185104, 47185106-08, 47185110-17

IX. ADDITIONAL REVIEWER'S COMMENTS: None.

DATA EVALUATION RECORD

HOMOBRASSINOLIDE TECHNICAL

STUDY TYPE: ACUTE ORAL TOXICITY - RAT (870.1100)
MRID 47185118

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 07-080

Primary Reviewer:
Susan Chang, M.S.

Signature:
Date: FEB 21 2008

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.

Signature:
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature:
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature:
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Acute Oral Toxicity - Rats (OPPTS 870.1100)
MRID NO:	47185118
DP BARCODE NO:	DP 347313
CASE NO:	Not reported
DECISION NO:	381556
TEST MATERIAL:	Homobrassinolide Technical (EPA Reg. No. 69361-RT, 86.3% 22S, 23S - homobrassinolide, a.i.)
PROJECT NO:	REPAR-HBR-TOX-19; IIBAT Study No. 06004
SPONSOR:	M/s. Godrej Agrovet Ltd., Pirojshanagar, Eastern Express Highway, Vikhroli, Mumbai – 400 079
TESTING FACILITY:	International Institute of Biotechnology and Toxicology (IIBAT), Padappai - 601 301, Kancheepuram District, Tamil Nadu, India
TITLE OF REPORT:	Homobrassinolide Technical, Biochemical Pesticides Toxicology Data, Acute Oral Toxicity in Wistar Rats
AUTHOR:	A. Sairam Kishore
STUDY COMPLETED:	March 28, 2006
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	The oral LD_{50} for female rats was greater than 5000 mg/kg.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY IV

I. STUDY DESIGN:

1. **Test Material**: Homobrassinolide Technical containing 86.3% 22S, 23S – homobrassinolide, a.i.

2. **Test Animals:** Three female Wistar rats were received from the animal house facility of IIBAT, Tamil Nadu, India and weighed 164-171 g on the day of dosing. The young adult animals, 8-12 weeks old, were housed individually in standard polypropylene rat cages with stainless steel top grills. The animals received standard rat pellet (M/s. tetragon Chemis Pvt. Ltd., Bangalore) and filtered water, *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 19.0-20.7°C; relative humidity, 50-64%; and photoperiod, 12 hour light/dark cycle. The air changes per hour were not reported.

3. **Methods:** Rats were ear-tagged: Nos. 1323, 1324, 1325 and were acclimated for five days and fasted overnight prior to dosing. The test material (5000 mg/kg body weight) in corn oil was dosed by gavage in two portions with a two hour interval between doses (Table 1). Forty eight hours after the first animal dosing, two additional animals were dosed. Body weight was recorded prior to dosing, and on days 7 and 14. The test animals were observed for clinical signs of toxicity 30 minutes, 1, 2, and 4 hours after dosing, and daily for 14 days. Morbidity and mortality were checked daily for 14 days. All animals were necropsied at the end of the study.

II. RESULTS:

1. **Mortality:** All rats survived the study.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg)	Males	Females	Combined
5000	-	0/3	-

Data taken from p. 18, Table 2, MRID 47185118.

2. **Body Weight:** All rats gained weight during the study.

3. **Clinical Observations:** No clinical signs of toxicity were observed during the study.

4. **Gross Necropsy:** No gross abnormalities were noted at necropsy.

III. DISCUSSION:

The oral LD_{50} for female rats was greater than 5000 mg/kg. This places Homobrassinolide Technical in TOXICITY CATEGORY IV. The packet classification is **ACCEPTABLE.**

DATA EVALUATION RECORD

HOMOBRASSINOLIDE TECHNICAL

STUDY TYPE: ACUTE DERMAL TOXICITY - RAT (870.1200)
MRID 47185120

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 07-080

Primary Reviewer:
Susan Chang, M.S.

Signature:
Date: FEB 21 2008

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.

Signature:
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature:
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature:
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Acute Dermal Toxicity - Rats (OPPTS 870.1200)
MRID NO:	47185120
DP BARCODE NO:	DP 347313
CASE NO:	Not reported
DECISION NO:	381556
TEST MATERIAL:	Homobrassinolide Technical (EPA Reg. No. 69361-RT, 86.3% 22S, 23S – homobrassinolide, a.i.)
PROJECT NO:	REPAR-HBR-TOX-21; IIBAT Study No. 06005
SPONSOR:	M/s. Godrej Agrovet Ltd., Pirojshanagar, Eastern Express Highway, Vikhroli, Mumbai – 400 079
TESTING FACILITY:	International Institute of Biotechnology and Toxicology (IIBAT), Padappai – 601 301, Kancheepuram District, Tamil Nadu, India
TITLE OF REPORT:	Homobrassinolide Technical, Biochemical Pesticides Toxicology Data, Acute Dermal Toxicity in Wistar Rats
AUTHOR:	A. Sairam Kishore
STUDY COMPLETED:	March 28, 2006
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	The dermal LD_{50} for males, females, and combined was greater than 2000 mg/kg.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY III

I. STUDY DESIGN:

1. **Test Material:** Homobrassinolide Technical containing 86.3% 22S, 23S – homobrassinolide, a.i.

2. **Test Animals:** Twenty male and 20 female Wistar rats were received from the animal house facility of IIBAT, Tamil Nadu, India were assigned, and weighed 283.6 g (male controls average weight), 190.60 g (female controls average weight), 280.2 g (treated males average weight), and 182.60 g (treated females average weight) on the day of treatment. The young adult animals, 8-12 weeks old, were housed individually in standard polypropylene rat cages with stainless steel top grills. The animals received standard rat pellet (M/s. tetragon Chemis Pvt. Ltd., Bangalore) and filtered water, *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 19.3-20.7°C; relative humidity, 52-62%; and photoperiod, 12 hour light/dark cycle. The air changes per hour were not reported.

3. **Methods**: Rats were ear-tagged: 1326-1330 (control males), 1331-1335 (control females), 1336-1340 (treated males), and 1341-1345 (treated females). The rats were acclimated for five days. The test material (2000 mg/kg body weight) moistened with a minimum volume of distilled water was applied over a 4 x 5 cm^2 area of the clipped dorsal trunk and covered with a gauze pad for 24 hours. The controls were treated with distilled water. The test animals were observed for clinical signs of toxicity daily for 14 days. Any reaction at the application site and change in fur was observed. The rats were weighed prior to treatment and on days 7 and 14. The rats were euthanized on day 14 and necropsied.

II. RESULTS:

1. **Mortality:** All rats survived the study.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg)	Males	Females	Combined
Control	0/5	0/5	0/10
2000	0/5	0/5	0/10

Data taken from p. 17, MRID 47185120.

2. **Clinical Observations:** No clinical signs of toxicity or dermal reaction at the application site were observed during the study.

3. **Body Weight:** The average body weight of all groups was increased at the end of the study.

4. **Gross Necropsy:** No test material related gross pathological abnormalities were noted at necropsy.

III. DISCUSSION:

The acute dermal LD_{50} for males, females, and combined was greater than 2000 mg/kg. This places Homobrassinolide Technical in TOXICITY CATEGORY III. The packet classification is **ACCEPTABLE.**

DATA EVALUATION RECORD

HOMOBRASSINOLIDE TECHNICAL

STUDY TYPE: ACUTE INHALATION TOXICITY - RAT (870.1300)
MRID 47185121

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 07-080

Primary Reviewer:
Susan Chang, M.S.

Signature:
Date: FEB 21 2008

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.

Signature:
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature:
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature:
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Acute Inhalation Toxicity - Rats (OPPTS 870.1300)
MRID NO:	47185121
DP BARCODE NO:	DP 347313
CASE NO:	Not reported
DECISION NO:	381556
TEST MATERIAL:	Homobrassinolide Technical (EPA Reg. No. 69361-RT, 86.3% 22S, 23S – homobrassinolide, a.i.)
PROJECT NO:	REPAR-HBR-TOX-22; IIBAT Study No. 06007
SPONSOR:	M/s. Godrej Agrovet Ltd., Pirojshanagar, Eastern Express Highway, Vikhroli, Mumbai – 400 079
TESTING FACILITY:	International Institute of Biotechnology and Toxicology (IIBAT), Padappai – 601 301, Kancheepuram District, Tamil Nadu, India
TITLE OF REPORT:	Homobrassinolide Technical, Biochemical Pesticides Toxicology Data, Acute Inhalation Toxicity in Wistar Rats
AUTHOR:	T. Kumar
STUDY COMPLETED:	March 15, 2006
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	The inhalation LC_{50} for males, females, and combined was > 2.26 mg/L.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY IV

I. **STUDY DESIGN:**

1. **Test Material:** Homobrassinolide Technical containing 86.3% 22S, 23S – homobrassinolide, a.i.

2. **Test Animals:** Ten male and ten female Wistar rats were received from the animal house facility of IIBAT, India, were assigned, and weighed 152.00 g (male controls average weight), 150.80 g (female controls average weight), 151.60 g (treated males average weight), and 150.80 g (treated females average weight) on the day of treatment. The young adult animals, 8-12 weeks old, were housed individually in standard polypropylene rat cages with stainless steel top grills. The animals received standard rat pellet (M/s. tetragon Chemis Pvt. Ltd., Bangalore) and filtered water, *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 19.6-24.2°C; relative humidity, 57-68%; and photoperiod, 12 hour light/dark cycle. The air changes per hour were not reported.

3. **Methods:** Rats were ear-tagged: Male – Nos. 1352 to 1356 (control) and 1362 to 1366 (treated); Female – Nos. 1357 to 1361 (control) and 1367 to 1371 (treated). The rats were acclimated for five days prior to exposure. The animals were exposed to the concentrations shown in Table 1. The rats were exposed head only using Rhema Labortechnik Inhalation equipment for four hours and observed daily for 14 days. They were weighed prior to test material exposure and on days 7 and 14. All rats were sacrificed and necropsied on day 14.

TABLE 1. Concentrations, exposure conditions, mortality/animals treated

Nominal Conc. (mg/L)	Grav. Conc. (mg/L)	MMAD (μm)	GSD (μm)	Particles ≤3.3 μm (%)	Temp (°C)	Humidity (%)	Mortality		
							Male	Female	Combined
-	Air only	-	-	-	19.6-21.3	59-60	0/5	0/5	0/10
ND	2.26	ND	ND	49-54	19.6-20.6	58-60	0/5	0/5	0/10

Data taken from Tables 2, 3, and 4, pp. 19, 20, and 21, MRID 47185121.
ND no data

Generation of the test atmosphere and description of the chamber: the inhalation exposure system consisted of a Demag air compressor, air filters, rotameters, pressure gauges, pressure regulators, flow regulators, a suction pump, impingers, a dust generator, a cyclindrical stainless steel inhalation chamber (60 L) and polyacrylic rat confinement cages. Three cylinders were placed one on top of the other. The upper cylinder had a stainless steel lid with a bore in the center for inserting the nebulizer. The middle cylinder was provided with 20 portholes with holders for fixing animal cages. The lower cylinder was for exhausting the chamber atmosphere.

Test atmosphere concentration: During exposure, samples were collected from one of the portholes of the inhalation chamber with Andersen 1 ACFM Non-viable Ambient Particle Sizing Samplers. The collection plates and backup filter were weighed separately. The total amount of particle collected on each stage was divided by the total amount collected to

determine the percentage of the total collected in each fraction. The total amount of particle collected was divided by the total volume of air samples from the inhalation chamber to determine the actual chamber concentration gravimetrically. The average results are in Table 1 above.

Particle size determination: Particle size distribution was estimated using an ambient particle sizing sampler (Anderson Series 20-800 1 ACFM Non-Viable Sampler). Results are in Table 1 above.

II. RESULTS:

1. **Mortality:** All rats survived the study.

2. **Clinical Observations:** No test material related signs of toxicity were noted from any animal.

3. **Body Weight:** Weekly mean body weight increased throughout the study.

4. **Gross Necropsy:** No test material related gross abnormalities were noted at necropsy.

III. DISCUSSION:

The inhalation LC_{50} for males, females, and combined was > 2.26 mg/L. This places Homobrassinolide Technical in TOXICITY CATEGORY IV. The packet classification is **ACCEPTABLE.**

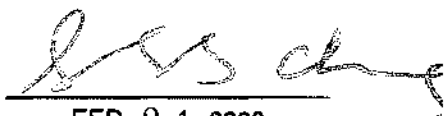
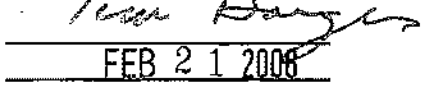
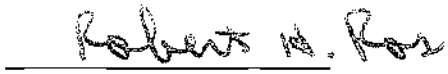
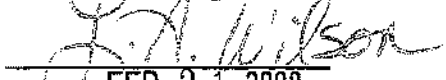
DATA EVALUATION RECORD

HOMOBRASSINOLIDE TECHNICAL

STUDY TYPE: PRIMARY EYE IRRITATION - RABBIT (870.2400)
MRID 47185122

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 07-080

Primary Reviewer: Susan Chang, M.S.	Signature: Date:	 FEB 21 2008
Secondary Reviewers: H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.	Signature: Date:	 FEB 21 2008
Robert H. Ross, M.S., Group Leader	Signature: Date:	 FEB 21 2008
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	 FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Acute Eye Irritation - Rabbits (OPPTS 870.2400)
MRID NO:	47185122
DP BARCODE NO:	DP 347313
CASE NO:	Not reported
DECISION NO:	381556
TEST MATERIAL:	Homobrassinolide Technical (EPA Reg. No. 69361-RT)
PROJECT NO:	REPAR-IIBR-TOX-23; IIBAT Study No. 8858
SPONSOR:	M/s. Godrej Agrovet Ltd., Pirojshanagar, Eastern Express Highway, Vikhroli, Mumbai – 400 079
TESTING FACILITY:	International Institute of Biotechnology and Toxicology (IIBAT), Padappai – 601 301, Kancheepuram District, Tamil Nadu, India
TITLE OF REPORT:	Homobrassinolide Technical, Biochemical Pesticides Toxicology Data, Eye Irritation in Himalayan Albino Rabbits
AUTHOR:	J. Baskaran
STUDY COMPLETED:	May 9, 2006
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	Corneal opacity was noted on 6/6 rabbits at one hour after test material instillation with resolution by day 7. Iritis was noted on 6/6 rabbits 24 hours after test material instillation with resolution by day 5. Positive conjunctival irritation (score 2 or 3) was noted on 1/6, 6/6, and 6/6 rabbits 1, 24, and 48 hours after test material instillation with resolution by 72 hours. The maximum average score was 41.33 at 24 hours after test material instillation. Homobrassinolide Technical was moderately irritating.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY III

I. STUDY DESIGN:

1. **Test Material:** Homobrassinolide Technical containing 53% active ingredient (as stated by the sponsor), the purity is 80.2% as stated on the Certificate of Analysis

2. **Test Animals:** Three male and three female Himalayan albino rabbits were received from the animal house facilities of IIBAT, India. The animals, age not reported, were housed individually in standard rabbit cages. The animals were fed standard pellet feed (M/s. Amrut Laboratory Animal Feed Ltd.) *ad libitum* and Aquaguard filtered water was available *ad libitum*. The animal room was air-conditioned with a 12 hour light/dark cycle. The temperature, relative humidity, and air changes per hour were not reported.

3. **Methods:** Rabbits were assigned Nos. 1 to 3 (males) and 4 to 6 (females) and weighed 1.40-1.70 kg on the day of treatment. The rabbits were acclimated for 7 days. The test material (0.1 mL = 52 mg/eye/animal) was applied in the conjunctival sac of the left eye of six rabbits. The right eye served as control. The eyes were examined and scored 1, 24, 48, 72, and 96 hours and at 5 and 6-7 days after test material instillation.

II. RESULTS:

1. **Mortality:** All rabbits survived the study.

2. **Ocular Lesions:** Corneal opacity was noted on 6/6 rabbits at one hour after test material instillation with resolution by day 6-7 (Table 1). Iritis was noted on 6/6 rabbits 24 hours after test material instillation with resolution by day 5 (Table 2). Positive conjunctival irritation (score 2 or 3) was noted on 1/6, 6/6, and 6/6 rabbits 1, 24, and 48 hours after test material instillation with resolution by 72 hours. The maximum average score was 41.33 at 24 hours after test material instillation (Table 3).

TABLE 1. Individual Male (M) and Female (F) Eye Scores w/ Time: Cornea (A=Density of Opacity, B=Area of Opacity)

Animal No.	1 hour		24 hours		48 hours		72 hours		96 hours		5 days		6-7 days	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1-M	1	1	2	2	2	2	2	2	2	2	1	1	0	0
2-M	1	1	2	2	2	2	2	2	2	2	1	1	0	0
3-M	1	1	2	2	2	2	2	2	2	2	1	1	0	0
4-F	1	1	2	2	2	2	2	2	2	2	1	1	0	0
5-F	1	1	2	2	2	2	2	2	2	2	1	1	0	0
6-F	1	1	2	2	2	2	2	2	2	2	1	1	0	0

Irritation score is based on Draize Method

TABLE 2. Summary of Eye Irritation Scores with Time: Conjunctiva and Iris							
Score Conditions	**1 hour**	**24 hours**	**48 hours**	**72 hours**	**96 hours**	**5 days**	**6-7 days**
Conjunctiva							
Erythema	1 to 2	3	2	1	0	0	0
Chemosis	1	3	2	1	0	0	0
Discharge	1 to 2	2 to 3	1 to 2	0 to 1	0	0	0
Iris	0	1	1	1	1	0	0

Irritation score is based on Draize Method

Scale for Scoring Ocular Lesions

Cornea

A. Opacity-degree of density (area most dense taken for reading)

No Opacity 0
Scattered or diffuse area, details of iris clearly visible 1*
Easily discernible translucent areas, details of iris slightly obscured 2*
Opalescent areas, no details of iris visible, size of pupil barely discernible 3*
Opaque, iris invisible 4*

B. Area of cornea involved

One quarter (or less) but not zero 1
Greater than one quarter, but less than half 2
Greater than half, but less than three quarters 3
Greater than three quarters, up to whole area 4
Score – A x B x 5 Total Maximum Score = 80

Iris

A. Values

Normal 0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive) 1*
No reaction to light, hemorrhage, gross destruction (any or all of these) 2*
Score = A x 5 Total Maximum Score = 10

Conjunctivae

A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal 0
Vessels definitely injected above normal 1
More diffuse, deeper crimson red, individual vessels not easily discernible 2*
Diffuse beefy red 3*

B. Chemosis

No swelling 0
Any swelling above normal (includes nictitating membrane) 1
Obvious swelling with partial eversion of lids 2*
Swelling with lids about half closed 3*
Swelling with lids about half closed to completely closed 4*

C. Discharge

No discharge 0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals) 1
Discharge with moistening of the lids and hairs just adjacent to lids 2

Discharge with moistening of the lids and hairs, and considerable area around the eye..... 3
Score = (A + B + C) x 2 Total Maximum Score = 20

* represents a positive response

TABLE 3. Summary of Total[a] and Primary Eye Irritation Scores with Time

Animal #	1 h	24 h	48 h	72 h	96 h	5 d	6-7 d
1-M	11	41	35	29	25	5	0
2-M	13	41	37	31	25	5	0
3-M	11	41	35	29	25	5	0
4-F	13	43	37	31	25	5	0
5-F	11	41	35	29	25	5	0
6-F	11	41	35	29	25	5	0
Average scores[b]	11.67	41.33	35.67	29.67	25.00	5.00	0.0

[a]Formula: Total Irritation Score = I + II + III, where,
I = Corneal Score = [Density (A) x Area (B)] x 5
II = Iris Score = Severity x 5
III = Conjunctival Score = [Erythema (A) + Chemosis (B) + Discharge (C)] x 2
[b]Average Primary Irritation = Sum of Total Irritation Scores ÷ 6

III. DISCUSSION:

Corneal opacity was noted on 6/6 rabbits at one hour after test material instillation with resolution by day 6-7. Iritis was noted on 6/6 rabbits 24 hours after test material instillation with resolution by day 5. Positive conjunctival irritation (score 2 or 3) was noted on 1/6, 6/6, and 6/6 rabbits 1, 24, and 48 hours after test material instillation with resolution by 72 hours. The maximum average score was 41.33 at 24 hours after test material instillation. Homobrassinolide Technical was moderately irritating and is in TOXICITY CATEGORY III. The packet classification is **ACCEPTABLE.**

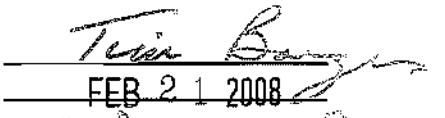
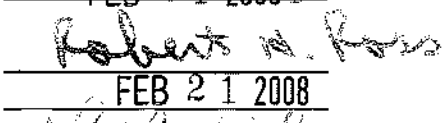
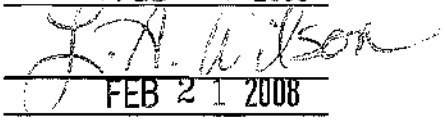
DATA EVALUATION RECORD

HOMOBRASSINOLIDE TECHNICAL

STUDY TYPE: PRIMARY DERMAL IRRITATION - RABBIT (870.2500)
MRID 47185123

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 07-080

Primary Reviewer: Susan Chang, M.S.	Signature: Date:	 FEB 2 1 2008
Secondary Reviewers: H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.	Signature: Date:	 FEB 2 1 2008
Robert H. Ross, M.S., Group Leader	Signature: Date:	 FEB 2 1 2008
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	 FEB 2 1 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Primary Dermal Irritation - Rabbits (OPPTS 870.2500)
MRID NO:	47185123
DP BARCODE NO:	DP 347313
CASE NO:	Not reported
DECISION NO:	381556
TEST MATERIAL:	Homobrassinolide Technical (EPA Reg. No. 69361-RT, 86.3% 22S, 23S – homobrassinolide, a.i.)
PROJECT NO:	REPAR-HBR-TOX-24; IIBAT Study No. 06006
SPONSOR:	M/s. Godrej Agrovet Ltd., Pirojshanagar, Eastern Express Highway, Vikhroli, Mumbai – 400 079
TESTING FACILITY:	International Institute of Biotechnology and Toxicology (IIBAT), Padappai – 601 301, Kancheepuram District, Tamil Nadu, India
TITLE OF REPORT:	Homobrassinolide Technical, Biochemical Pesticides Toxicology Data, Primary Skin Irritation in New Zealand White Rabbits
AUTHOR:	A. Sairam Kishore
STUDY COMPLETED:	March 28, 2006
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	No dermal irritation was noted on any rabbit. The primary irritation index was 0.0. Homobrassinolide Technical was non-irritating.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY IV

I. STUDY DESIGN:

1. **Test Material:** Homobrassinolide Technical containing 86.3% 22S, 23S homobrassinolide, a.i.

2. **Test Animals:** Three female young adult New Zealand White rabbits were received from the animal house facility of IIBAT, Tamil Nadu, India and weighed 2.4-2.9 kg on the day of treatment. The animals, age not reported, were housed individually in standard rabbit cages. The animals received standard pellet feed (M/s. Amrut Laboratory Animal Feed) and filtered water, *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 18.7-19.5°C; relative humidity, 49-61%; and photoperiod, 12 hour light/dark cycle. The air changes per hour were not reported.

3. **Methods:** Rabbits were assigned Nos. 4041 to 4043 and were acclimated for five days. The fur on the dorsal trunk of each rabbit was clipped on the day prior to treatment. The rabbits were treated with 0.5 g of test material moistened with minimum volume of distilled water applied on a 6 cm^2 clipped intact dose site, and the site covered with a gauze patch. The patch was loosely held in contact with the skin using a semi-occlusive dressing. The covering was removed 4 hours later and the site cleansed to remove any residual test material. The animals were observed for clinical signs of toxicity during the study. Dermal examination was recorded at 1, 24, 48, and 72 hours after removal of the patch.

II. RESULTS:

1. **Mortality:** All rabbits survived the study.

2. **Dermal responses:** No dermal irritation was noted on any rabbit. The primary irritation index was 0.0.

Irritation Scores:

TABLE 1. Summary of individual rabbit's dermal irritation scores with time

	Hours			
Animal Nos.	1	24	48	72
4041	0/0[a]	0/0	0/0	0/0
4042	0/0	0/0	0/0	0/0
4043	0/0	0/0	0/0	0/0

Data taken from Table 1, p. 16, MRID 47285123.

[a]Erythema/Edema

Description of rating method:

Evaluation of Skin Reaction:	Score
Erythema formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Edema Formation:

No edema 0
Very slight edema (barely perceptible) 1
Slight edema (edges of area well-defined by definite raising) 2
Moderate edema (raised approximately 1 mm) 3
Severe edema (raised by more than 1 mm extending beyond the area of exposure) 4

III. DISCUSSION:

No dermal irritation was noted on any rabbit. The primary irritation index was 0.0. Homobrassinolide Technical was non-irritating and is in TOXICITY CATEGORY IV. The packet classification is **ACCEPTABLE.**

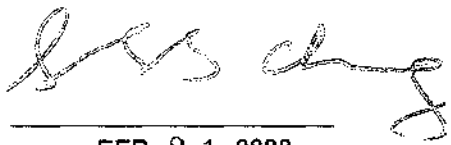
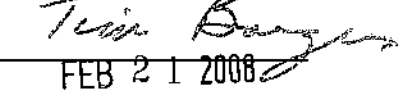
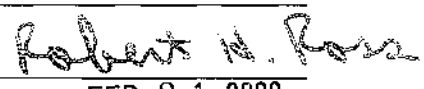
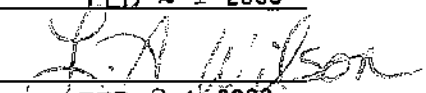
DATA EVALUATION RECORD

HOMOBRASSINOLIDE TECHNICAL

STUDY TYPE: SKIN SENSITIZATION - GUINEA PIG (870.2600)
MRID 47185124

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 07-080

Primary Reviewer: Susan Chang, M.S.	Signature: Date:	 FEB 21 2008
Secondary Reviewers: H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.	Signature: Date:	 FEB 21 2008
Robert H. Ross, M.S., Group Leader	Signature: Date:	 FEB 21 2008
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	 FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Skin Sensitization - Guinea Pigs (OPPTS 870.2600)
MRID NO:	47185124
DP BARCODE NO:	DP 347313
CASE NO:	Not reported
DECISION NO:	381556
TEST MATERIAL:	Homobrassinolide Technical (EPA Reg. No. 69361-RT, 22S, 23S – homobrassinolide, a.i.)
PROJECT NO:	REPAR-HBR-TOX-25; IIBAT Study No. 11656
SPONSOR:	M/s. Godrej Agrovet Ltd., Pirojshanagar, Eastern Express Highway, Vikhroli, Mumbai – 400 079
TESTING FACILITY:	International Institute of Biotechnology and Toxicology (IIBAT), Padappai – 601 301, Kancheepuram District, Tamil Nadu, India
TITLE OF REPORT:	Homobrassinolide Technical, Biochemical Pesticides Toxicology Data, Skin Sensitization Potential in Guinea Pigs
AUTHOR:	J. Baskaran
STUDY COMPLETED:	May 9, 2006
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	After three consecutive weekly inductions, the test and control animals showed no signs of reactivity at 24 and 48 hours after challenge. The study did not include a positive control study which was carried out within six months of the study. Homobrassinolide Technical would not be a dermal sensitizer if the registrant provides a positive control study carried out within six months of the study and the results are appropriate.
CLASSIFICATION:	**UNACCEPTABLE, but upgradable** if the registrant provides a positive control study which was carried out within six months of the study and the results are appropriate.

I. STUDY DESIGN:

1. **Test material:** Homobrassinolide Technical containing 45% active ingredient (as stated by the sponsor), the purity is 80.2% as stated on the Certificate of Analysis

2. **Test animals:** Thirty-five male guinea pigs (source not specified) were assigned to groups and weighed 300-320 g at experiment start. The animals, age not reported, were housed individually in standard polypropylene cages. The animals were fed with carrots, tomatoes, cabbage, and soaked chickpeas and were supplemented with ascorbic acid (200 mg/kg diet). Clean filtered well water was available *ad libitum*. The animal room was air-conditioned with a 12 hour light/dark cycle. The temperature, relative humidity, and air changes per hour were not reported.

3. **Methods:** The male guinea pigs were assigned numbers and grouped: Pilot study (three animals); Test – Nos. 1 to 20; Control – Nos. 21 to 30. The guinea pigs were quarantined for 14 days and acclimated for 5 days. The animals were induced and challenged according to the method of Buehler. The flank area of 20 test guinea pigs and 10 control animals were clipped prior to each treatment. For the induction, 500 mg test material moistened with a minimum of distilled water was applied to a cotton pad about 4-6 cm^2 in size and placed on the shaven area of the animal and secured with an occlusive patch and bandage dressing. The patch and dressing were removed after six hours and excess test material removed. The control animals were treated with distilled water only. The procedure was repeated once each week for three consecutive weeks. On day 28, the test and control animals were challenged with 250 mg test material moistened with a minimum of distilled water under occlusion to the clipped posterior flank for 6 hours. In addition, all animals were treated with distilled water at an anterior site for 6 hours. Reactions were scored at 30 and 54 hours following challenge application.

II. RESULTS:

1. **Mortality:** All animals survived the study.

2. **Body Weight:** Body weight was not reported.

3. **Skin Effects:** No skin reaction was noted on any animal after challenge.

TABLE 1. Summary of Individual Erythema Challenge Scores with Time [a]

	24 hours				48 hours			
Erythema Score	0	1	2	3	0	1	2	3
Treated	20	0	0	0	20	0	0	0
Control	10	0	0	0	10	0	0	0

[a]Number of animals affected
Evaluation score is based on Magnusson and Kligman Grading Scale.

Scale for Scoring Skin Reaction

Magnusson-Kligman scoring scale

	Score
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

III. DISCUSSION:

After three consecutive weekly inductions, the test and control animals showed no signs of reactivity at 24 and 48 hours after challenge. The study did not include a positive control study which was carried out within six months of the study. Homobrassinolide Technical was not a dermal sensitizer if the registrant provides a positive control study which was carried out within six months of the study and the results were appropriate. The packet is classified as **UNACCEPTABLE without a validation of the test procedure.**

DATA EVALUATION RECORD

HOMOBRASSINOLIDE TECH

STUDY TYPE: ACUTE ORAL TOXICITY - MOUSE (870.1100)
MRID 47208903

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 07-080

Primary Reviewer:
Susan Chang, M.S.

Signature:
Date: FEB 21 2008

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.

Signature:
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature:
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature:
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Acute Oral Toxicity – Mice (OPPTS 870.1100)
MRID NO:	47208903
DP BARCODE NO:	DP 347313
CASE NO:	Not reported
DECISION NO:	381556
TEST MATERIAL:	Homobrassinolide Technical (EPA Reg. No. 69361-RT, 86.3% 22S, 23S homobrassinolide, a.i.)
PROJECT NO:	REPAR-HBR-TOX-20; JRF Report No. 0640
SPONSOR:	Godrej Soaps Limited, Pirojshanagar, Eastern Express Highway, Vikhroli, Bombay – 400 079
TESTING FACILITY:	Jai Research Foundation, Valvada 396 108, District Valsad, Gujarat, India
TITLE OF REPORT:	Homobrassinolide Technical, Biochemical Pesticides Toxicology Data, Acute Oral Toxicity in Mice
AUTHOR:	P.B. Deshmukh
STUDY COMPLETED:	November 1993
GOOD LABORATORY PRACTICE:	OECD GLP Compliant
CONCLUSION:	The mouse oral LD_{50} for male, female, and combined was greater than 5000 mg/kg.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY IV

I. STUDY DESIGN:

1. **Test Material**: Homobrassinolide Technical, Batch No. 001093, 80.2% purity

2. **Test Animals:** Twenty-five male and 25 female Swiss mice were received from the animal house of Jai Research Foundation and weighed 22.5-29.0 g (test material dosed males) and 23.0-30.0 g (test material dosed females) on the day of dosing. The young adult animals, 6-7 weeks old, were housed in groups in plastic cages with wiremesh tops. The animals received standard mice feed (Lipton India Limited, Bangalore) and water, *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 26-29°C; relative humidity, 67-90%; and photoperiod, day/night cycle. The air changes per hour were not reported.

3. **Methods:** Mice were identified by cages and color codes and were acclimated for seven days and fasted overnight prior to dosing. The test material (0, 1500, 2500, 3500, or 5000 mg/kg body weight) in distilled water was dosed by gavage (Table 1). Body weight was recorded prior to dosing, 24 hours post dosing, and on days 7 and 14. The test animals were observed for abnormal toxic symptoms hourly for 6 hours on the first day, twice on the second day, and daily during the remainder of the study. Mortality was checked.

II. RESULTS:

1. **Mortality:** All mice survived the study.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg)	Males	Females	Combined
Control	0/5	0/5	0/5
1500	0/5	0/5	0/5
2500	0/5	0/5	0/5
3500	0/5	0/5	0/5
5000	0/5	0/5	0/5

Data taken from p. 17-20, MRID 47208903.

2. **Body Weight:** All mice dosed with the test material gained weight during the study. No report was given for the control mice.

3. **Clinical Observations:** No symptoms were observed during the study.

4. **Gross Necropsy:** No necropsy data were reported.

III. DISCUSSION:

The oral LD_{50} for male, female, and combined mice was greater than 5000 mg/kg. This places Homobrassinolide Technical in TOXICITY CATEGORY IV. The packet classification is **ACCEPTABLE.**

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for Hypersensitivity Incidents (OPP 152-16)

MRID 47185131

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T.

Signature: Sylvia Milanez
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for Hypersensitivity Incidents (OPP 152-16)
MRID NO:	47185131
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-TOX-41
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. Immune Response.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	The information submitted is sufficient to support the requested waiver for hypersensitivity incidents, although any future incidents must still be reported to the Agency per 40 CFR 158.690(c).

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for Hypersensitivity Incidents (OPP 152-16).

Registrant's Justification

Results of acute studies with Homobrassinolide Technical are summarized in Table 1. Based on those studies, the registrant found the test material to be practically non-toxic, with no human health problems expected. None of the test animals demonstrated any immediate or delayed reaction manifested by skin rash, shortness of breath, generalized swelling, and/or tearing. It is therefore unlikely that Homobrassinolide Technical would cause any allergenic reactions leading to immediate or delayed hypersensitivity. As a result, the registrant does not believe that precautionary labeling regarding eye and skin exposure is needed for Homobrassinolide Technical or end use products containing the active ingredient. Additionally, virtually no human exposure is expected after products containing homobrassinolide are applied to target crops.

Table 1. Acute toxicity of Homobrassinolide Technical

Oral LD_{50} (rat)	MRID 47185118	>5000 mg/kg	Toxicity Category IV
Oral LD_{50} (mouse)	MRID 47208903	>5000 mg/kg	Toxicity Category IV
Dermal LD_{50} (rat)	MRID 47185120	>2000 mg/kg	Toxicity Category IV*
Inhalation LC_{50} (rat)	MRID 47185121	2.26 mg/L	Toxicity Category IV
Eye irritation (rabbit)	MRID 47185122	Mild irritant	Toxicity Category III
Skin irritation (rabbit)	MRID 47185123	Not an irritant	Toxicity Category IV
Skin sensitization (guinea pig)	MRID 47185124	Not a sensitizer**	--

*Registrant's classification. ORNL reviewer notes this should be Toxicity Category III

**ORNL reviewer classified this study as unacceptable, but upgradable upon submission of an acceptable positive control study

Reviewer's Conclusion

The information submitted is sufficient to support the requested waiver for hypersensitivity incidents, although any future incidents must still be reported to the Agency per 40 CFR 158.690(c). The registrant's statement that precautionary labeling is not needed for end use products containing the active ingredient may not be valid, depending on what inert ingredients are contained in the end use product(s).